Title Potato Seed Tuber Physiological Age and Tolerance of Attack by the Potato Cyst Nemotode Globodera Pallida

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Potato seed tuber physiological age and tolerance of attack by the potato cyst nematode *Globodera pallida*

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1990

A thesis submitted to the Council for National Academic Awards in partial fulfilment of the requirements for the degree of Doctor of Philosophy

Luton College of Higher Education

(sponsoring establishment)

Rothamsted Experimental Station

Cambridge University

(collaborating establishments)

To Biddy

for her support and encouragement

during my research studentship

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ACKNOWLEDGEMENTS

Many people have helped me during my three years as a research student at Rothamsted and I wish to take this opportunity to thank them:-

Rothamsted

Ken Evans for supervision and encouragement during research and thesis preparation. I am also grateful for his practical help with field trials and for word-processing this thesis.

Mike Russell and Corinna Flynn for their assistance with field work.

Alan Todd and Gina Smith for the statistical analysis of experimental data.

Mike Robinson and Paul Burrows for their advice and instruction in the techniques of molecular biology.

Members of the Photography Department for the preparation of plates.

Other staff at Rothamsted who have helped me.

Luton College of Higher Education

Ken Robertson, Gordon Storey and Graham Steele for their administrative work within the college and with the CNAA.

Cambridge University

Supervisor Eric Allen and staff at the University Farm for their assistance with the physiological ageing of potato seed tubers.

Green's of Soham Ltd

For providing the trial sites in 1987 and 1988. Bill Smith for his helpful cooperation with field trials at Rosedene Farm.

DECLARATION

While registered as a candidate for the degree of Doctor of Philosophy for the C.N.A.A., the author has not been a registered candidate or enrolled student for another award of the C.N.A.A. or other academic or professional institution during the research programme. No material contained in this thesis has been used in any other submission for an academic award.

.....(Candidate)

.....(Supervisor)

ABSTRACT

P. P. J. Haydock PhD Thesis 1990

POTATO SEED TUBER PHYSIOLOGICAL AGE AND TOLERANCE OF ATTACK BY THE POTATO CYST NEMATODE G. PALLIDA

Seed tubers conditioned to 0, 200 or 400 day-degrees above 4°C were grown in land infested with G. pallida. Plants from 400 day-degree seed emerged earlier, had larger canopies and greater dry weights early in the growing season than plants from 0 day-degree seed. However, physiological ageing reduced peak percentage ground cover and advanced crop senescence so that similar quantities of solar radiation were intercepted over the whole growth period. Overall, total and ware yields were not affected much by seed tuber physiological age. The partially resistant cultivars tested were more tolerant than the non resistant cultivars but tolerance was not usually affected by physiological age of seed tubers. The effects of nematicide treatment, initial G. pallida population density, cultivar maturity class, cultivar resistance status and planting date on nematode multiplication, plant growth and tolerance of attack by G. pallida are discussed.

Using data from a variable temperature water bath experiment, probability and regression analysis estimated mean basal temperatures for the development of G. pallida and G. rostochiensis at 2.5 and 5.1°C; least variance analysis estimates were 3.5 and 4.1°C respectively. Approximately 200 day-degrees above 3.5 and 4.1°C were required from the inoculation of JJ2 of G. pallida and G. rostochiensis to the peak numbers of JJ5 found in potato roots.

From a range of chemicals tested for their ability to release antigen from G. pallida cysts, sodium hypochlorite was found to be the most effective. Released antigen was detected using polyclonal antisera and monoclonal antibodies in an ELISA test. The potential for the development of an ELISA based diagnosis test for PCN using species-specific antibodies is discussed.

CHAPTER 1

AN INTRODUCTION TO THE POTATO CROP AND POTATO CYST NEMATODES

CHAPTER 1 AN INTRODUCTION TO THE POTATO CROP AND POTATO CYST NEMATODES

1.1 THE POTATO CROP

1.1.1 History

The modern European potato belongs to the species Solanum tuberosum ssp. tuberosum. It is probable that S. tuberosum ssp. andigena was brought into Europe from South America in the late sixteenth century. Unsuited to the long summer daylengths of Europe, tuber yields were poor and S. tuberosum ssp. andigena was regarded as little more than a botanical curiosity. Strong artificial selection for earliness of tuber formation from the late sixteenth to the late eighteenth century resulted in the genetic shift of subspecies andigena to the European subspecies tuberosum. By the mid eighteenth century cultivars adapted to long summer photoperiods were in widespread cultivation as a field crop throughout Europe (Hawkes, 1982).

1.1.2 Production and Usage

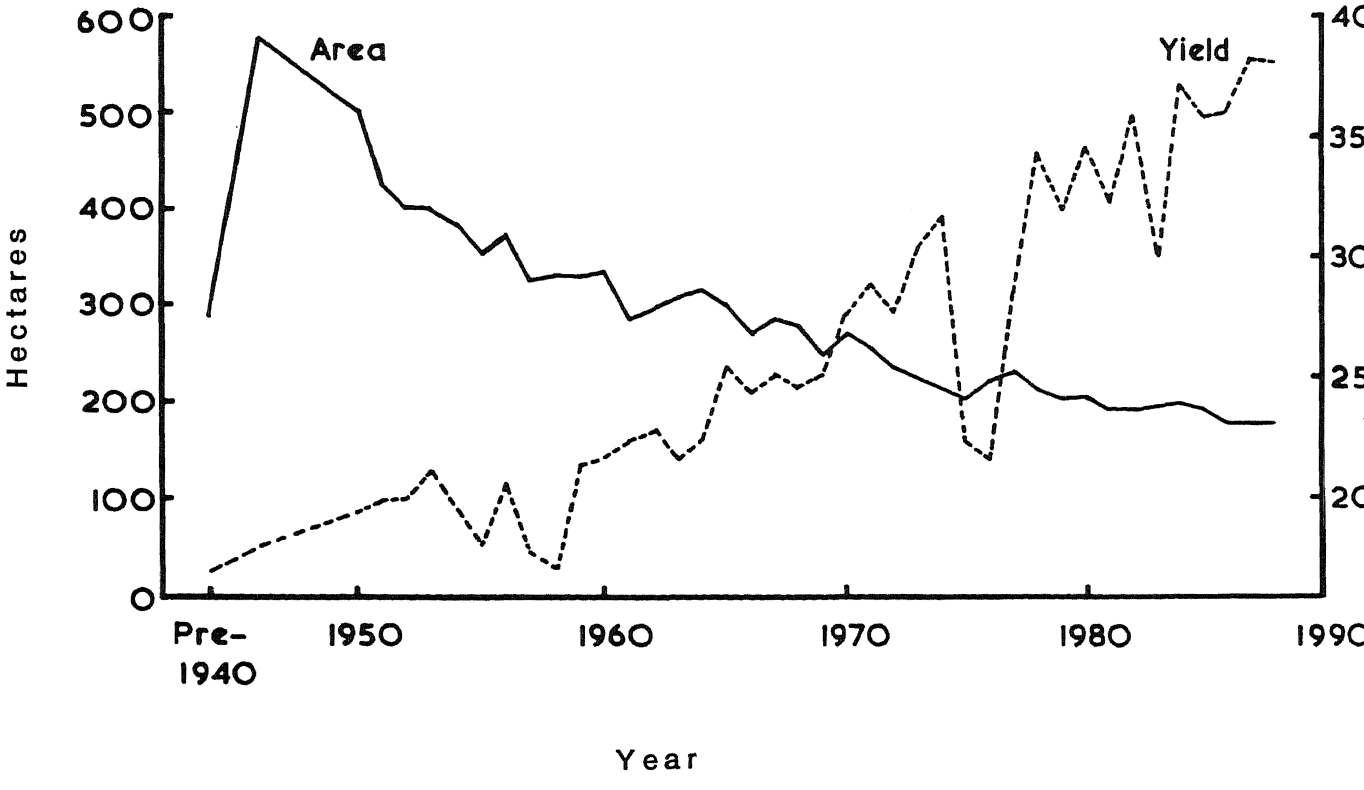
Production

In Great Britain 178,600 ha of potatoes were grown in 1987 yielding 6.76M tonnes of tubers. With an average price of £85.76/tonne paid to producers the crop was worth £534M and Britain was 89% self sufficient (Anon. 1989a). Since 1948 the area of potatoes planted has decreased as average yields have risen to approximately 38t/ha (Fig. 1).

Usage

In 1987 95% of tuber production in Great Britain was for human consumption. The British eat an average of 114 kg/head/annum, which is the largest per capita consumption in Europe (Potato Marketing Board, (a) undated). The remaining domestic usage is accounted for by seed tubers, compensation and stockfeed buying programmes, chats, waste and retained stockfeed

Fig.1 Area grown and average yields of potatoes (all maturity classes) for the period 1946-1988 (From Evans and Haydock, 1990)



(Anon., 1989).

Seed tuber production

In the U.K., seed potatoes are produced by specialist growers in areas of low aphid incidence. These regions include northern and eastern Scotland, Northern Ireland and areas of northern England. Producers aim to grow a high yield of 32-60 mm tubers which are true to type and free from viruses, pests and disease. Seed tubers are grown for further muliplication (VTSC, virus tested stem cuttings; SE, super elite; E, elite) or for ware production (CC, certified seed). In 1987 a total of 77,000 tonnes of seed tubers were exported from Scotland (Anon. 1989).

1.1.3 Potential Yield and Canopy Growth

Estimates of the maximum potential yield of maincrop potatoes grown in Europe range from 90t/ha (Alcock, 1967) to 115t/ha (Allen and Scott, 1980). A potato "blueprint" was drawn up to test whether the theoretical yield of 90t/ha calculated by Alcock could be achieved in practice in the U.K. Physical and management inputs were set at high levels so that the only factors limiting growth were climatic. Using the blueprint, yields of 90t/ha were achieved (Gunn, 1978). Potential tuber yield is probably nearer 115t/ha than 90t/ha as yields of over 100t/ha have been obtained without altering inputs (E. J. Allen, pers. comm.).

Monteith (1977) found a close relationship between total annual dry matter yield and the amount of radiation intercepted by the crop canopy. Burstall and Harris (1983) reported a positive linear relationship between the easily measured percentage ground cover and the percentage of incident light intercepted. The efficiency of conversion of incident radiation to dry matter is similar for potatoes, sugar-beet, apples and barley at 1.4 g dry matter/MJ intercepted solar radiation. Linear

relationships between both total and tuber dry matter yields and the amount of radiation intercepted by potato crops have been reported by Allen and Scott (1980).

Early in the growing season much incident radiation is wasted as it falls on bare ground. Canopy production depends upon the rates of crop emergence, leaf initiation and leaf expansion. Crop growth rate is maximum at the "critical leaf area index" of 3-5 in potatoes (Scott and Bremner, 1966; Bremner and Taha,1966) when the canopy is intercepting approximately 95% of incident solar radiation. Structural differences in the canopy can affect yield. Trenbath and Angus (1975) showed that higher yields are often achieved with more erect leaves as light distribution throughout the canopy is more uniform and photosynthesis in the lower leaves is less limited by reduced light intensities. As the crop matures crop growth rates decline rapidly because older leaves are less photosynthetically efficient, the proportion of respiring plus non-photosynthetic tissue increases and leaf area index decreases due to senescence (Wright, 1986).

It is important that changes in leaf area index match the seasonal variation in incident solar radiation so that the maximum quantity of solar radiation can be intercepted. Cereals reach peak leaf area in June and July when light intensity, temperature and therefore potential net assimilation rates are highest. Potatoes reach their peak leaf area index much later when the environment is becoming less conducive to photosynthesis (Watson, 1947). Gunasena and Harris (1969) showed that there is a correlation between tuber yield and leaf area duration throughout the whole of the growth period. Allen and Scott (1980), in their review of potato crop growth, concluded that when pests and diseases are controlled and water supply is adequate the most important objective is to maximise radiation interception.

1.1.4 Maturity Class

Potatoes can be classified as early, second early or maincrop cultivars according to certain growth characteristics which influence their potential harvest period.

First Early Cultivars

First earlies, e.g. Maris Bard, are grown most successfully in soils such as sandy loams which are light in texture and warm up early in the year. These tend to be in coastal areas (with mild winters) in the west of England and Wales e.g. Cornwall and Pembrokeshire.

Seed tubers are usually sprouted (physiologically aged) during storage prior to planting which can accelerate the growth cycle of the potato plant by 10-14 days compared with unsprouted seed tubers. Planting starts as soon as soil conditions permit, which is usually in February or early March, and continues to early April. Approximately 3.2-4.5t/ha of seed tubers are planted into narrow (54-66cm) rows. Harvesting starts in late May, with yields of 7.5-10t/ha of uniform, small tubers. Premium prices are paid for the first harvested earlies. Prices fall as yields increase into June. If left to maturity first early cultivars would yield less than maincrop cultivars because first earlies have smaller canopies which intercept less solar radiation.

Second Early Cultivars

Second earlies, e.g. Estima, are best grown in the same warm, light textured soils as first early cultivars. They are planted immediately after the first earlies but are harvested later giving yields of up to 50-60t/ha by the end of August. Second earlies provide a continuous supply of new potatoes through to the maincrop harvest.

Maincrop Cultivars

Maincrop cultivars, e.g. Maris Piper, Pentland Dell, Sante and Morag, are often grown in deep, moisture retentive soils

throughout Britain. Growers aim to have their seed tubers planted by mid April in southern England and late April in northern England and Scotland. Seed rates vary according to growing conditions and cultivar. The cultivar King Edward requires 1.5-2.3t/ha of seed tubers whilst Desiree and white skinned cultivars producing large tubers need rates of 3.2-4.5t/ha, depending on seed size. Traditionally, maincrops have been grown in 71cm wide rows. Currently 76, 80 or 90cm rows are common because they allow easier mechanical access for cultural operations and result in less tuber damage and greening. Tubers are initiated later in maincrop than in early and second early cultivars, allowing greater foliage growth. This results in good crops yielding 40-80t/ha when harvested in October.

1.1.5 Fertiliser

The quantities of the major nutrients nitrogen, phosphate and potash that are required by a potato crop depend upon soil type, previous cropping and the maturity class of the crop. Early potatoes generally require less nitrogen and potash than maincrops. In 1982 average fertiliser applications to maincrop potatoes were 190kg N/ha, 192kg P_2O_5/ha, 237kg K_2O/ha and 38% of the crops received extra nutrients from farmyard manure (Johnson, 1984). Potatoes respond well to fertiliser but a 40t/ha crop will only remove 40kg P_2O_5/ha and 232kg K_2O/ha. Most crops leave residual phosphate and potash which can be utilised by the following crop in the rotation.

1.1.6 Cultivation and Weed Control

Potatoes require a deep, moist, friable, clod free seed bed. Ploughing to 200-300mm depth in the autumn allows the soil to weather during the winter. Spring preparation aims to avoid soil compaction and clod formation. Using caged or double wheels on

machinery to decrease ground pressure, seed beds are prepared with the minimum number of passes over the land. The majority of planting is carried out mechanically.

Weeds can be controlled prior to crop emergence by levelling and re ridging as required. Alternatively, contact herbicides such as paraquat can be sprayed onto the undisturbed ridges. After crop emergence periodic, careful inter row cultivation or the protection of pre-emergent residual herbicides are required until the potato canopies have met between the rows. In early crops and high rainfall areas, weed control is mainly achieved using herbicides (Toosey, 1986). Approximately 65-70% of potato fields are treated with herbicides (Lutman, 1984).

1.1.7 Diseases

The potato crop is subject to a number of diseases, most of which are capable of causing serious losses of yield and quality. The most important diseases of leaves and stems are potato blight (fungus: *Phytophthora infestans*), potato leaf roll virus (vector: peach potato aphid *Myzus persicae*), potato virus Y (vector: *Myzus persicae*), potato virus X, blackleg (*Erwinia carotovora* var. *atroseptica*) and spraing (potato mop top virus, vector: powdery scab fungus). Tubers can be affected by common scab (*Streptomyces scabies*), gangrene (*Phoma exigua* var. *foveata*), dry rot (*Fusarium solani* f.sp. *caeruleum*) and powdery scab (*Spongospora subterranea*).

Control of most diseases is achieved by the combination of resistant varieties, the use of certified seed and the adoption of appropriate husbandry practices. Crop protection chemicals are important in the control of potato blight and some tuber diseases (Anon. 1984).

1.1.8 Pests

Pests of potatoes may reduce the productivity of the plants

by direct feeding damage or by transmitting virus diseases. Alternatively they may disfigure the tubers, thereby reducing their market value. The most effective pest control measures are usually applied prior to or at the time of planting. Pests include the aphid Myzus persicae, the garden slug Arion hortensis, wireworms Agriotes spp., cutworms Agro tis segetum and the free living nematodes Trichodorus and Paratrichodorus spp. However, the most important pests of the potato crop are the potato cyst nematodes.

1.2 POTATO CYST NEMATODES (PCN)

1.2.1 Origin and Introduction into Europe

The potato cyst nematodes Globodera pallida and G. rostochiensis are the most important nematode pests of potatoes. Both species were introduced to Europe from Andean regions of South America in the 1850's. They were probably imported along with potato collections used in breeding programmes for late blight resistance (Evans and Brodie, 1980). PCN went unnoticed until 1881 when they were observed on potatoes in Germany. Records suggest that they were present in Britain by 1900 or earlier (Jones, 1970).

1.2.2 Distribution

PCN are widespread throughout the world; Evans and Stone (1977) reported the presence of PCN in 48 countries.

In Britain, PCN can be found in all the major potato growing areas. Globodera pallida and G. rostochiensis often occur in mixed populations with the latter predominating in East Anglia, the South East, the West Midlands and Scotland (Jones and Kempton, 1982). However, Hancock (1988) reports soil sampling by ADAS to have revealed that G. pallida is increasingly important in some areas.

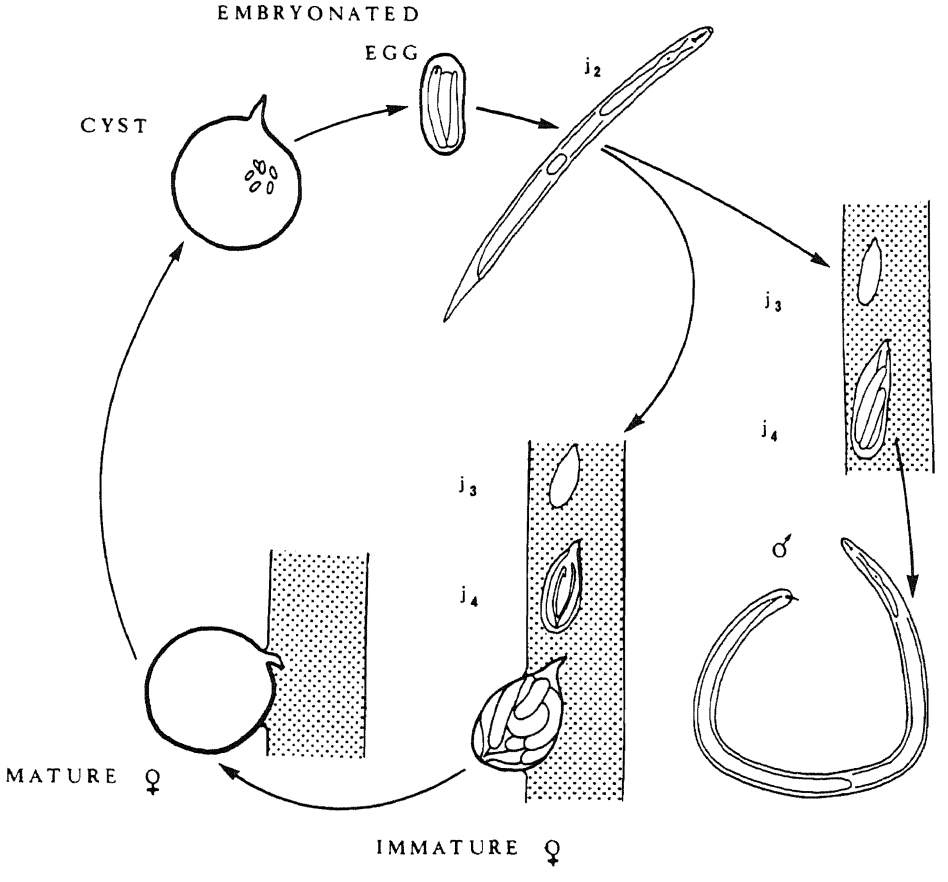
1.2.3 Biology and Life Cycle

Differences between populations of PCN had been reported since 1966 (Guile, 1966,1967,1970; Evans and Webley, 1970) and it was eventually realised that two species were involved (Jones, Carpenter, Parrott, Stone and Trudgill, 1970). However, there are few biological differences between G. pallida and G. rostochiensis (Stone, 1973).

PCN are among the most highly specialised and successful of all the plant parasitic nematodes (Evans and Stone, 1977). Females are sessile and globose while males are motile and vermiform. Adults are preceded by four juvenile stages. The second stage juvenile (J2) is both the dormant and infective stage of the PCN's life cycle (Fig. 2). The J2 juvenile overwinters in an egg which together with 200-500 other eggs is protected by a tough cyst. Each cyst is the tanned remains of a mature female's cuticle.

In the spring, J2's hatch from the eggs, usually in response to hatching factors exuded from potato roots (Clarke and Perry, 1977). These root exudates stimulate 60-80% of viable juveniles to hatch (Jones, 1970). The hatched J2 enters the root of a host plant just behind the root tip or at a lateral root and migrates through the root, away from the tip, by piercing cell walls with its stylet. This leaves a trail of ruptured cells leading to the stele, where the J2 stops and begins to feed on pericycle, cortex or endodermis cells. Cell walls are pierced and the contents withdrawn via the hollow spear-like stylet. Feeding induces the modification of host cells to form a large syncytial transfer cell. This transfer cell has a dense, granular cytoplasm with wall ingrowths which increase the surface area for nutrient transfer from the plant to the cell. The nematode remains feeding at the transfer cell until it reaches maturity (Jones and Northcote, 1972). The sex of the developing nematode is

Fig.2 Life cycle of Globodera rostochiensis and G.pallida



(From Evans and Stone,1977)

influenced by nutrition (Trudgill, 1967) and can be visually determined at the J3 juvenile stage. After the final moult, adult males emerge from the J3 cuticle. They do not feed and only remain active for about 10 days (Evans, 1970). J4 females enlarge and eventually rupture the root cortex, exposing their bodies for mating. Adult males are chemically attracted to receptive females. PCN are amphimictic and both males and females may mate many times (Green, Greet and Jones, 1970).

1.2.4 Pathotypes

The "international" pathotype scheme for PCN proposed by Kort, Ross, Rumpenhorst and Stone (1977) recognised three pathotypes of *G. pallida* and five of *G. rostochiensis*. Of these, Ro1, Pa1, Pa2, and Pa3 are known from the U.K. (Stone, Holliday, Mathias and Parrott, 1986). However, there are many problems associated with the use of pathotype schemes and these are discussed with host resistance in Chapter 2.

1.2.5 Plant Damage and Economic Cost

PCN injure potato roots and stunt their growth, resulting in a diminished supply of water and nutrients to the stems and leaves. They also directly remove nutrients from the roots (Stone, 1973). Evans and Stone (1977) reported that PCN cause annual losses equivalent to approximately 9% of U.K. production. For the 1987 season this would represent a loss of £48M of revenue to growers. Using the results of advisory tests for PCN population densities between 1982 and 1986, Hancock (1988) calculated that, if no action were taken, yield losses of approximately 6% of production or £36M (using 1987 production figures) would occur. However, this is not the full cost of PCN to the economy. Legislation, chemical control, enforced rotation and the maintenance of a resistance breeding programme are control measures, each with their own costs, which have to be

employed because of PCN.

1.2.6 PCN Management

Legislation, crop rotation, chemical control and resistant cultivars can be used together as an integrated pest management strategy.

Legislation

The Potato Cyst Eelworm (Great Britain) Order 1973 requires that land on which commercial seed is grown is free from PCN (Anon. 1982). Official soil examination confirms the presence or absence of PCN in a field. This order is intended to prevent the spread of PCN cysts to other ware growing areas.

Crop Rotation

With an annual rate of PCN population decline in the absence of a host crop of 33%, it could take 12 years between susceptible potato crops for the nematode population density to decline from 1000 eggs/g of soil to less than 10 eggs/g of soil. This length of crop rotation is unacceptably long to commercial potato growers: a rotation with 4-6 years between potato crops will result in a worthwhile decline in PCN populations (Whitehead, 1986).

Chemical Control

Chemical control of PCN in the U.K. relies on two oxime-carbamate nematicides. Aldicarb ("Temik") and oxamyl ("Vydate") are nematostatic cholinesterase inhibitors (Evans, 1973). These chemicals were applied to approximately 40,000ha of potato growing land in England and Wales in 1985 (Whitehead, 1986). At £230/ha this represents a cost of £9.2M to potato growers. Nematicides are less effective in soils where *G. pallida* dominates a mixed population or where *G. pallida* is present on its own (Whitehead, Tite, Fraser and Nicholls, 1984). This may be due to the length of hatching period being greater for *G. pallida*

than for G. rostochiensis (Whitehead, 1986).

Resistance

Resistance in potato plants reduces the number of females that develop in the root system (Philips, 1984) and is measured in terms of the nematode's multiplication rate i.e. the ratio of final (Pf) to initial (Pi) population density in the soil. Cultivars with single gene (H1) resistance to G. rostochiensis pathotypes Ro1 and Ro4 have been available since the late 1960's. These cultivars, for example Maris Piper, have been bred from Solanum tuberosum ssp. andigena (CPC 1673) X S. tuberosum hybrids. Repeated use of these cultivars in land infested with both G. rostochiensis and G. pallida has selected out G. pallida (Whitehead, 1986). Single dominant gene resistance to G. pallida is not available in commercial cultivars. Partial resistance to G. pallida, conferred by several genes, has been bred into the two commercially available cultivars, Morag and Sante, which are currently being evaluated by the National Institute of Agricultural Botany (NIAB).

The efficacy of integrating control methods is illustrated for G. rostochiensis by the work of Jones (1969). He looked at varying combinations of fallow and host crop, resistant Maris Piper and non resistant Pentland Dell, and the use of fumigation with the nematicide dazomet (Table 1). When all three methods were used in sequence, 99.85% of the nematodes were killed. If a non resistant cultivar was then grown, and the nematode mutiplication rates were X30 or X70, it would only increase the nematode population to 4.5 or 10.5% of the initial population respectively.

The integrated control of G. rostochiensis using soil applied granular nematicides, crop rotation and resistant cultivars has reduced yield losses attributable to this species to approximately 2% per annum (Anon. 1983).

TABLE 1 Integration of control methods for G. rostochiensis (from Jones, 1969)

Control method(s)	Resulting population (% initial population)	Kill (%)	Population after growing and harvesting a susceptible cultivar, calculated at two nematode multiplication rates* (% of initial population)	
			30 X	70 X
1 4 years, no potatoes	3	97	90	> 100
2 1 year, resistant crop	20	80	> 100	> 100
3 Nematicide (fumigant)	25	75	> 100	> 100
4 1 and 2	0.6	99.4	18	42
5 1 and 3	0.75	99.25	22.5	52.5
6 2 and 3	5	95.0	> 100	> 100
7 All three methods	0.15	99.85	4.5	10.5

*The observed maximum reproductive rate is thought to lie between 25 and 75 times but could be greater.

CHAPTER 2

RESEARCH OBJECTIVES AND LITERATURE REVIEW

CHAPTER 2 RESEARCH OBJECTIVES AND LITERATURE REVIEW

2.1 RESEARCH OBJECTIVES

1) To investigate the effects of seed tuber physiological age on the growth and tolerance of potato cultivars to attack by the potato cyst nematode *Globodera pallida*.

Section 2.2 explains the concepts of resistance and tolerance and examines the use of resistant and tolerant cultivars in PCN infested land. The effects of seed tuber physiological age on the growth and yield of the potato plant are discussed in Section 2.3.

2) To investigate the effect of temperature on the life cycle of *G. pallida* and *G. rostochiensis*.

Research into the relationship between environmental temperature and PCN development along with its practical application is reviewed in Section 2.4.

3) To investigate the practicality of developing a quantitative immunoassay to estimate PCN population densities in infested soil.

Section 2.5 describes the production of monoclonal antibodies/polyclonal antisera and their utilisation in the enzyme linked immunosorbent assay (ELISA).

2.2 RESISTANCE AND TOLERANCE

2.2.1 Introduction

As a consequence of basic differences between viruses, bacteria, fungi, insects and nematodes, standardised definitions cannot be used throughout all the branches of plant pathology. However "resistance" is generally used to describe the ability of the host to resist or hinder pathogen invasion, development or multiplication while "tolerance" describes the extent to which

the host is able to withstand infection without suffering damage (Trudgill, 1986a).

With PCN, even resistant plants are invaded, so tolerance is not dependent on resistance. Resistance and tolerance are both frequently less than absolute and are capable of varying continuously (Dropkin, 1955). While these concepts will be considered separately, the variation and extremes of tolerance and resistance are illustrated in Fig. 3.

2.2.2 Resistance

Terminology

Veech (1981) defined resistance as the absence or inhibition of disease development upon challenge by a pathogenic agent. Therefore, anything that prevents, retards or restricts the development of disease contributes to host resistance. A resistant host prevents the completion of the nematode's life cycle within the plant's root system. A partially resistant cultivar allows less multiplication than a non-resistant host.

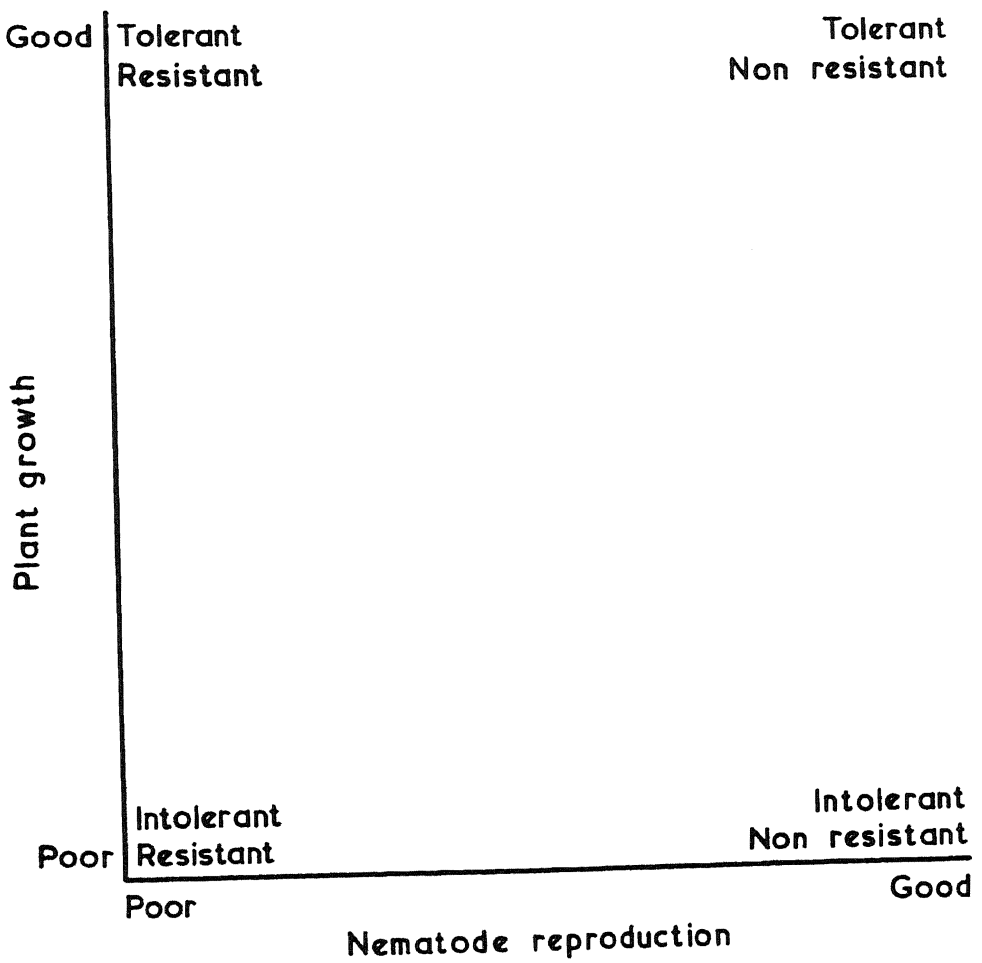
Resistance levels are often expressed as a ratio of final to initial nematode population densities (Pf/Pi), but because genotype x environment interactions can affect the absolute value of this multiplication rate, a more useful figure is the multiplication rate relative to that obtained with a standard non-resistant cultivar.

Pathotypes are races of a nematode species distinguished by an inherited ability or inability to reproduce on specified lines of a host plant species that embody different genes for resistance to the nematode. Virulence is the ability of a nematode to break resistance and reproduce on a resistant host (Stone, 1985).

History

In 1952 Ellenby discovered resistance to PCN in several

Fig.3 A diagramatic representation of the independence of resistance and tolerance in plants, with all combinations of the two characteristics possible (From Evans and Haydock, 1990)



lines of cultivated S. tuberosum ssp. andigena and in wild S. vernei, which caused the nematode-induced syncytium to degenerate and the juvenile either to die or to develop into a male (Hoopes, 1977). In the initial tests, S. tuberosum andigena allowed very little nematode reproduction but nematode populations were subsequently found that were able to reproduce unhindered (Jones, 1957; Dunnett, 1957; Jones and Pawelska, 1963). Careful studies on a range of nematode populations revealed that there were two sibling species of potato cyst nematodes, G. rostochiensis and G. pallida (Stone, 1973).

Pathotypes

Kort et al. (1977) proposed their "international" pathotyping scheme for PCN (Table 2) to provide a common system to replace the separate Dutch, German and British schemes in operation prior to 1977 (Table 3). The "international" scheme recognised five pathotypes of G. rostochiensis (Ro1 to Ro5) and three of G. pallida (Pa1 to Pa3). Of these, Ro1, Pa1, Pa2 and Pa3 were known in the U.K. (Stone et al. 1986).

To identify a pathotype it must be defined against a known resistance gene. In the current "international" scheme only three of the six differential clones used have identified resistance genes (Table 4), which makes the scheme scientifically unsound. Pathotypes Pa2 and Pa3 of G. pallida and Ro2, Ro3 and Ro5 of G. rostochiensis are defined by their reproductive ability on differential clones of unknown genetic constitution and it is assumed that these pathotypes are in reality artefacts of the test procedure (Stone, 1985).

Parrott (1981) provided evidence of a gene-for-gene relationship governing interactions between G. rostochiensis pathotype Ro1 and gene H1 derived from S. tuberosum ssp. andigena and G. pallida pathotype Pa1 and gene H2 from S. multidissectum. Genetic proof for a gene-for-gene relationship has been provided

Table 2 Kort et al.'s "international" pathotype scheme

National pathotypes.....		British A Dutch A Hiltrup	Dutch B Obersteinbach	Dutch C	Dutch F	Harmerz	British B	Dutch D	Frenswegen Chavorney Dutch E British E
New pathotypes.....		Ro1	Ro2	Ro3	Ro4	Ro5	Pa1	Pa2	Pa3
Clone	Plant Resistance Code								
S. tuberosum ssp. *tuberosum*		+	+	+	+	+	+	+	+
S. tuberosum ssp. *andigena* CPC 1673 hybr.	Ro1, 4	—	+	+	—	+	+	+	+
S. kurtzianum hybr. 60.21.19	Ro1, 2	—	—	+	+	+	+	+	+
S. vernei hybr. 58.1642/4	Ro1, 2, 3	—	—	—	+	+	+	+	+
S. vernei hybr. 62.33.3	Ro1, 2, 3, 4 Pa1, 2	—	—	—	—	+ —	—	—	+
S. vernei hybr. 65.346/19	Ro1, 2, 3, 4, 5	—	—	—	—	—	+	+	+
S. multidissectum hybr. P 55/7	Pa1	+	+	+	+	+	—	+	+
S. vernei hybr. 69.1377/94	Ro1, 2, 3, 4, 5 Pa1, ,2, 3	—	—	—	—	—	—	—	—

Table 3 Former British and Dutch pathotype schemes

British	A	B	E
Solanum tuberosum ssp. *tuberosum*	+	+	+
S. tuberosum ssp. *andigena* CPC 1673 hybr.	—	+	+
S. multidissectum hybr. P55/7	+	—	+

Dutch	A	B	C	D	E	F
S. tuberosum ssp. *tuberosum*	+	+	+	+	+	+
S. tuberosum ssp. *andigena* CPC 1673 hybr.	—	+	+	+	+	—
S. kurtzianum hybr. 60.21.19 *	—	—	+	+	+	+
S. vernei hybr. 58.1642/4 *	—	—	—	+	+	+
S. vernei hybr. 62.33.3 *	—	—	—	—	+	—

* Formerly designated KTT 60-21-19, G-LKS 58.1642/4 and $(VT^n)^2$ 62.33.3. We have dropped the initial letters to shorten the clone names.

(From Kort et al., 1978)

Table 4 Pathotyping scheme for PCN after Kort et al. (1978) with known genes for resistance indicated

Clone	Resistance gene(s)	Pathotype Ro1	Ro2	Ro3	Ro4	Ro5	Pa1	Pa2	Pa3
S. tuberosum ssp. *tuberosum*	None	+	+	+	+	+	+	+	+
S. tuberosum ssp. *andigena* CPC 1673 hybrid	H_1	−	+	+	−	+	+	+	+
S. kurtzianum hybrid 60.21.19	*A*, *B*	−	−	+	+	+	+	+	+
S. vernei hybrid 58.1642/4	?	−	−	−	+	+	+	+	+
S. vernei hybrid 62.33.3	?	−	−	−	−	±	−	−	+
S. vernei hybrid 65.346/19	?	−	−	−	−	−	+	+	+
S. multidissectum hybrid P55/7	H_2	+	+	+	+	+	−	+	+

?, polygenes or complex of major and minor genes.
+, $Pf/Pi > 1{\cdot}0$.
−, $Pf/Pi < 1{\cdot}0$.

(From Stone et al., 1986)

for the H1 resistance gene in S. tuberosum ssp. andigena CPC 1673 by Janssen, Bakker and Gommers (in press).

In a review of the current pathotyping scheme, Trudgill (1985) concluded that as the important sources of resistance are mainly polygenic or oligogenic it would be difficult to produce an effective scheme encompassing all types of resistance. It is important to recognise that variation exists in both the nematode and the potato and to devise more reliable methods of measuring it.

Resistance in Available Cultivars

Despite problems with pathotyping, breeders quickly produced valuable cultivars incorporating the ex andigena gene H1. The most widely grown of these is the maincrop cultivar Maris Piper which was grown on 26,700ha of land in Great Britain in 1984 (Anon. 1985). The H1 resistance gene provides effective control of all British G. rostochiensis populations (Alphey, Phillips and Trudgill, 1988).

Major gene H2 resistance derived from S. multidissectum is available in genotypes imparting resistance to G. pallida Pa1. The main sources of resistance to G. pallida Pa2/3 are S. vernei and S. tuberosum ssp. andigena. Cultivars with partial resistance derived from S. vernei also inherit partial resistance to G. rostochiensis. Genotypes from S. tuberosum ssp. andigena have partial resistance to pathotype Pa2/3 but no resistance to G. rostochiensis. Resistance to G. pallida is partial (or incomplete) because only some of the polygenes are inherited (Dale and Phillips, 1982). There are currently no cultivars with full resistance to G. pallida Pa2/3 (Alphey, et al. 1988).

The National Institute for Agricultural Botany (Anon. 1989b) list seven "recommended" and eight "non-recommended" cultivars with Ro1 resistance (Table 5). The "provisionally recommended" cultivar Sante has both Ro1 and partial Pa2/3 resistance and is

TABLE 5 NIAB listed cutivars with PCN resistance

Cultivar	Status					Resistance	
	R	PRO	PRO (SU)	NR	IT	RO1	PA2/3
Pentland Javelin	X					X	
Cara	X					X	
Kingston	X					X	
Maris Piper	X					X	
Aminca		X				X	
Costella		X				X	
Sante			X			X	PR
Fronika				X		X	
Penta				X		X	
Skirza				X		X	
Alhamra				X		X	
Jewel				X		X	
Morene				X		X	
Saturna				X		X	
Ausonia				X		X	
Alcmaria					X	X	
Concorde					X	X	
Rocket					X	X	PR
Dundrod					X	X	
Premiere					X	X	
Nadine					X	X	PR
Stroma					X	PR	PR
Waregem					X	PR	PR
Navan					X	X	
Stemster					X	X	
Cultra					X	X	
Gigant					X	X	
Morag					X	PR	PR

R = recommended

PRO = provisionally recommended

PRO(SU) = provisionally recommended for special use

NR = non recommended

IT = in trial

PR = partially resistant

(after Anon, 1989b)

listed for "special use" as part of an integrated PCN management system. In NIAB trials there are 13 cultivars with partial or full Ro1 resistance, including five with partial resistance to Pa2/3 (Table 5), so it is likely that more cultivars with partial Pa2/3 resistance will achieve recommended status in the future.

The Use of Partial Resistance

Granular nematicides such as aldicarb rarely give adequate control of G. pallida when used in conjunction with non-resistant cultivars (Whitehead, Tite, Fraser and Nicholls, 1984,1987; Phillips, Dale, Green, Hancock, Holliday, Lacey and Tones, 1988). Where partially resistant cultivars are grown in nematicide treated soil there is usually a reduction in the number of nematodes with the most resistant cultivars reducing population density to the greatest extent.

Until agronomically acceptable cultivars are bred with higher levels of resistance than is currently available, the combination of partial resistance and nematicide is the best method for reducing populations of G. pallida (Phillips et al. 1988). If a reasonable length of time between potato crops is allowed, then lower inputs of nematicides may be required to achieve control of G. pallida when partially resistant cultivars are grown (Trudgill, Tones and Mathias, 1985; Alphey, Phillips and Trudgill, 1988). As nematicides are both expensive and highly toxic, this would be a positive contribution to environmental conservation (Phillips and Dale, undated).

2.2.3 Tolerance

The degree of tolerance of a potato plant may be expressed as the yield loss of a test cultivar relative to a standard cultivar under the same conditions of nematode challenge. Evans and Trudgill (1978) defined tolerant cultivars as those which suffer a smaller reduction in yield at high nematode densities.

Tolerance by potato plants of cyst nematode attack has been reviewed in depth by Evans and Haydock (1990).

Plant Growth and Tolerance

The most obvious effect of PCN on potato plant growth is the stunting of the root system and the water stress that this causes. The potato is a drought sensitive species and yield can be drastically reduced by severe water stress. Evans and Franco (1979) suggest that differences in the tolerance of PCN between cultivars are due to differences in the efficiency of water use; cultivars that are best able to tolerate nematodes are those which use water most efficiently (Evans, 1982a). Cara is more tolerant than Pentland Dell because it has a larger root system permitting a more complete exploitation of soil water reserves, and can prevent excessive water loss by rapid stomatal closure (Fatemy et al. 1985). Shoot:root ratios are usually consistent between cultivars at a given nematode population density but tend to decrease with increasing initial population density (Evans, 1982b).

Tolerance in U.K. Cultivars

There is much evidence to show that British potato cultivars differ in their ability to tolerate attack by PCN. Evans (1982b) showed that in heavily infested plots the yield of Cara was reduced by only 4.8t/ha compared with 24.6t/ha for Pentland Crown and 32.9t/ha for Pentland Dell. Other examples are listed by Trudgill (1986b) and include those of Whitehead et al. (1980), Trudgill et al. (1983), Trudgill and Cotes (1983a) and Trudgill, Mathias and Tones (1985). However, in trials conducted by Brown (1983) and Brown and Sykes (1983) no evidence for differences in tolerance of PCN attack between cultivars could be found.

The Use of Tolerance

Where potatoes are grown in PCN infested land the yield potential of the crop is reduced and the choice of a tolerant

cultivar increases potential yield above that of an intolerant cultivar of the same maturity class. Cultivars such as Maris Piper with the H1 resistance gene and a high degree of tolerance of attack by G. rostochiensis have been available since the late 1960's (Howard, 1970). Multigenic resistance to G. pallida derived from S. vernei has been incorporated into new cultivars but these tend to be intolerant of nematode attack. It is important that new cultivars are screened for both their resistance and tolerance status, a process which has been carried out at the National Institute of Agricultural Botany since 1984 (Gurr, 1987).

The choice of a tolerant cultivar as part of an integrated PCN management programme will enable the profitability of a potato crop grown in infested land to be maximised.

2.3 POTATO SEED TUBER PHYSIOLOGICAL AGE

Potato growers have for many years recognised that the duration and temperature of seed tuber storage prior to planting has an important effect on crop growth and tuber yield (Hartmann, 1934). Kawakami (1952) described the stage of development of a seed tuber as its "physiological age". He found that the greater sprout growth associated with increasing physiological age resulted in accelerated haulm production. Divergence from the optimum ("proper") physiological age, by using seed that was too "young" or too "old", reduced tuber yield (Kawakami, 1962). Toosey (1964a,b) reviewed the effects of pre-sprouting seed tubers, emphasising the practical importance of physiologically ageing tubers in storage prior to planting.

2.3.1 Measurement of Physiological Age

In order to research, compare the results from research and exploit the potential benefits of manipulating seed tuber physiological age, an accurate and widely accepted method of

quantifying this process had to be found. Research with this aim can be divided into two areas; firstly the biochemical changes occurring in seed tubers associated with physiological ageing and secondly temperature accumulation by the seed tuber during storage prior to planting.

Biochemical Changes in the Seed Tuber

After harvest seed tubers are in the "rest period" of their development, during which time they will not sprout even if placed in optimum environmental conditions. The rest period lasts from 5-19 weeks depending upon the cultivar (Emilsson, 1949). After the rest period there is a phase of true dormancy during which sprout growth will commence if conditions are favourable. True dormancy can last for 18-33 weeks but is usually in the 20-23 week range (Madec and Perennec, 1969). These periods of tuber rest and true dormancy are caused by the changing proportions of growth hormones such as gibberellins, auxins and abscisins (Perennec and Madec, 1980). Rappaport and Wolf (1969) stated that gibberellic and abscisic acids probably determine the length of the rest period.

Frenkel (1978) found that hydrogen peroxide levels in the tuber rise with increasing physiological age. Peroxidase concentration in the tuber may be indicative of physiological age (Es and Hartmans, 1987).

When dormancy is broken, nucleic acid and protein sythesis increase. Apelbaum (1984) suggests that the enzyme ornithine decarboxylase (part of the polyamine pathway essential for protein production) could be used as an indicator of physiological age.

Reust and Aerny (1985) analysed the citric acid, malic acid and sucrose content of seed tubers. During storage and sprouting the concentrations of sucrose and malic acid increased whilst that of citric acid decreased.

None of the biochemical changes discussed have been used practically to provide quantitative estimates of seed tuber physiological age.

Accumulated Day-Degrees

Both the "incubation period" (Claver, 1951) and "sprouting capacity" (Krijthe, 1962) have been proposed as potentially useable measurements of seed tuber physiological age (van der Zaag and Loon, 1987). However, it is the concept of accumulated day-degrees that has received most attention.

Wurr (1978a) showed that the length of the longest sprout and total sprout length were the most useful tuber characteristics to quantify sprout development. Temperature accumulation from the start of sprout growth was suggested as a measure of physiological age expressed as the sum of the daily mean potato store temperatures above a base of 0 °C (O'Brien and Allen, 1978). Wurr (1978b) found a linear relationship between total sprout length per tuber and accumulated day-degrees above 0 °C (calculated from dormancy break) which accounted for more than 90% of the variation in sprout length occurring in the cultivars Pentland Crown, Majestic and Desiree. He demonstrated that there was a significant relationship between the yield of a ware crop and physiological age of the seed tubers. This only occurred when storage temperature had a large effect upon yield (Wurr, 1978c). Bean and Allen (1980) proposed that physiological age was more correctly estimated as accumulated day-degrees above 4 °C after the break of dormancy, taken as the production of a 3mm long sprout (Plate 1). O'Brien, Allen, Bean, Griffith, Jones and Jones (1983) used field trial data to determine the most appropriate method of calculating accumulated day-degrees. This involved varying the base temperature and starting point of calculation, followed by the regression of tuber yields on the various scales produced. The best fit was obtained using the

Plate 1 Seed-tuber physiological age and sprout growth

0 day-degrees (at break of dormancy)

400 day-degrees

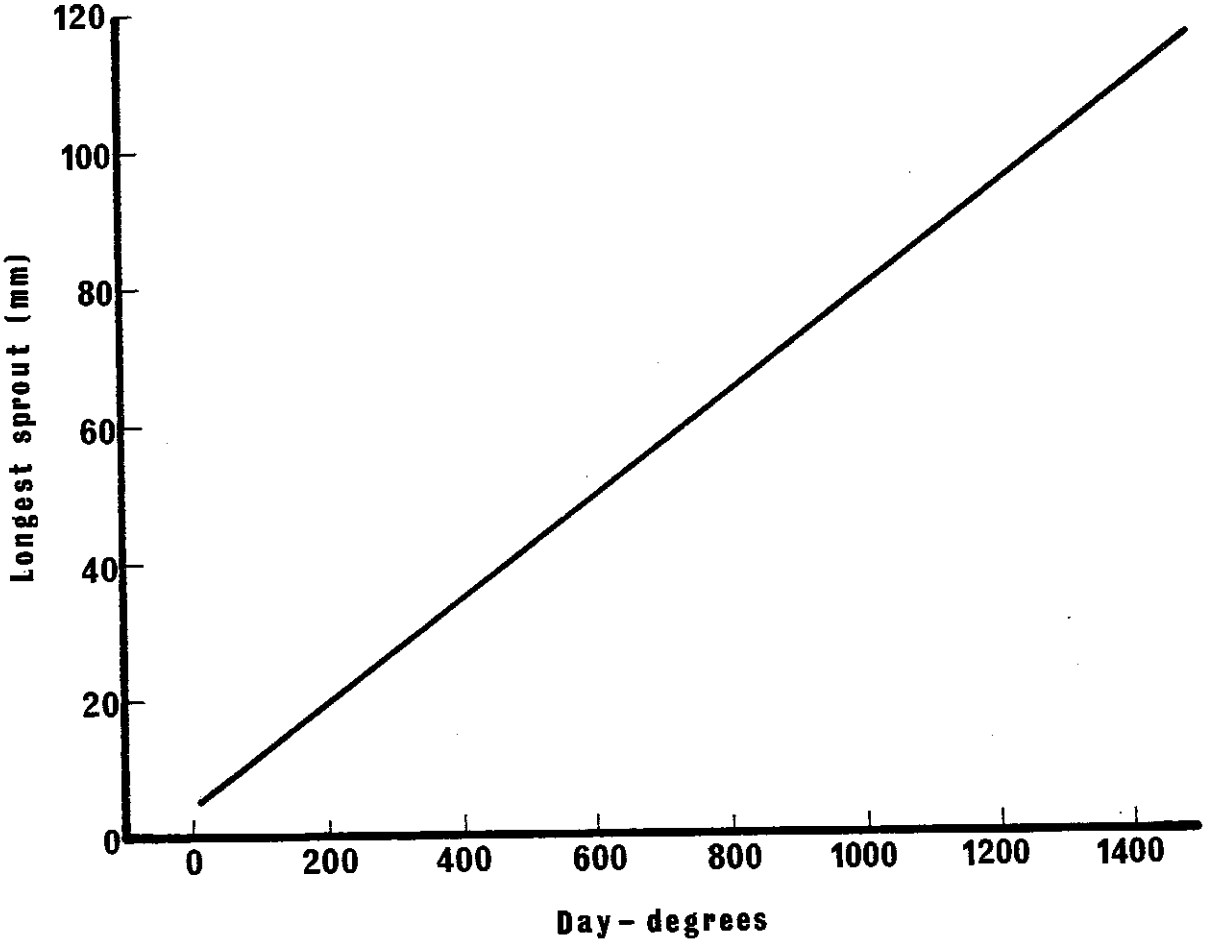
length of the longest sprout and the number of day-degrees above 4 °C after the onset of sprout growth (Fig.4). The positive linear relationship between length of the longest sprout on a tuber and accumulated day-degrees above 4 °C was confirmed by Allen and O'Brien (1986) and is widely used as a practical measure of seed tuber physiological age. However, there are several inconsistencies in the practical use of this method that need to be discussed.

Krijthe (1962) reported that even when seed was kept at temperatures which were too low for sprouting to occur, for example 2 °C, physiological development still proceeded. O'Brien and Allen (1984) showed that desprouting removes the effects of physiological age, which implies that these effects are carried in the sprout and not in the tuber.

The base temperature of 4 °C used in calculations was chosen because most cultivars will not grow or have limited sprout growth at this temperature (O'Brien et al. 1983). Some cultivars, for example Home Guard (Raouf, 1979), will grow at 4 °C but the basal temperature for sprout growth has not been accurately determined for any cultivar (O'Brien et al. 1983). Therefore it is probable that physiological age is being underestimated for cultivars which sprout below 4 °C and overestimated for those which will only grow at temperatures above 4 °C.

Calculation of physiological age starts when the tuber has broken dormancy. Krijthe (1962) used the same criterion as Emilsson (1949) and Schepers (1956) and considered that dormancy had ended when at least 80% of tubers in a batch had developed sprouts which were 3mm in length. Wurr (1978a, 1979) interpreted dormancy break as being when the total sprout length per tuber was 2mm. The end of the dormant period was defined by Panelo and Caldiz (1989) as being when 80% of tubers bore sprouts at least 5mm long. Reust and Aerny (1985) assumed dormancy to have ended

Fig.4 Relationship between length of the longest sprout per tuber and the number of day-degrees above 4°C



(adapted from O'Brien et al. 1983)

when 80% of tubers had produced sprouts 3mm long. However, most workers consider dormancy to have ended when an individual tuber has a sprout which is 3mm in length or when 90% of tubers in a batch have sprouts at least 3mm long (Bean and Allen, 1980; Anon. 1981; O'Brien et al. 1983; Davies, 1984; Allen and O'Brien, 1986; Burstall and Harris, 1986; O'Brien et al. 1986; Gillison, Jenkins and Hayes, 1987). To enable the comparison of experimental results it is important that a standard definition of dormancy break is agreed upon and used.

It is assumed that the rate of progression of the physiological ageing process is linearly related to temperature. This suggests that the manner in which physiological age is accumulated should not affect the final outcome. For example a tuber conditioned to 500 day-degrees over a long period at a low temperature should be physiologically identical to a tuber aged at a higher temperature for a shorter period provided that both tubers have accumulated the same number of day-degrees. Wurr (1979) found that tubers conditioned using different temperature regimes but to the same physiological age could have significantly different total sprout lengths per tuber. Gillison et al. (1987) in a similar investigation found that crops grown from seed tubers which had accumulated their physiological age late in the storage period grew in a manner characteristic of physiologically older seed. They suggested that the calculated number of day-degrees underestimates the seed tuber's physiological age the nearer the period of ageing is to planting.

2.3.2 Physiological Age and Growth of the Potato Plant

When seed tubers are physiologically aged prior to planting, the following changes in their growth occur:-

1) Earlier plant emergence

2) Earlier tuber initiation

3) Fewer tubers are set in most cultivars

4) Plant size is reduced in some cultivars resulting in reduced tuber bulking rates

5) Plants are more susceptible to water stress

6) Senescence occurs earlier in most cultivars

7) Optimum nitrogen applications are increased

(Potato Marketing Board,(b) undated)

Plant Emergence

Headford (1962) reported that increasing sprout length up to 1.5cm considerably shortened the time taken for the crop to emerge. Sprouting to over 1.5cm in length had relatively little effect. With Maris Bard conditioned to 800 day-degrees above 4 °C, 90% emergence occurred almost 3 weeks earlier than with unconditioned seed tubers (O'Brien et al. 1983). For Home Guard with 1400 day-degrees this difference was 2 weeks (O'Brien et al. 1986). Allen and O'Brien (1986) also found that physiologically ageing Pentland Javelin seed tubers hastened emergence.

In India, Sinha (1974) and Jain and Sinha (1982) reported accelerated plant emergence from physiologically aged seed tubers. Sprouting for 1-4 weeks hastened crop emergence while sprouting for 7 weeks resulted in decreased emergence. Northern India experiences maximum temperatures of 30-36 °C during the sprouting period in September which probably caused excessive shrinking and loss of viability in the 7 weeks sprouting treatment (Sinha and Rai, 1982).

Vakis (1986) increased the rate of emergence of Arran Banner, Cara and Spunta in Cyprus by physiologically ageing the seed tubers at ambient temperatures.

Madec and Perennec (1962) and Bus and Schepers (1978) found that physiological age had no effect on plant emergence. The findings of Fischnich and Krug (1963) were the same but it is important to realise that they used resprouted seed i.e. seed

tubers from which all the sprouts were removed and then the tubers physiologically aged.

Loon (1987) reported later emergence from physiologically aged seed. This was probably caused by using excessively old seed. Emergence is slow from seed tubers that are either excessively young or old (Madec, 1978).

Tuber Initiation

The onset of tuber initiation and rate of tuber growth are both advanced by increasing physiological age of the seed tubers (Madec and Perennec, 1959;1962). In conditions which are favourable for crop growth this occurs only up to a maximum sprout length of 1.5cm (Headford, 1962; Headford and Ingersent, 1962). O'Brien et al. (1983) showed that the number of days from planting to the mean date of tuber initiation decreased linearly with increasing physiological age for the cultivars Home Guard and Maris Bard, a relationship confirmed by O'Brien et al. (1986) who found a difference of 3.5 to 4 weeks in Home Guard with 1400 day-degrees compared with unconditioned seed tubers.

Physiological age is a major determinant of the timing of tuber initiation in potatoes (O'Brien et al. 1983).

Number of Tubers

Allen et al. (1979) and O'Brien et al. (1986) reported that the number of tubers per plant generally decreased with increasing seed tuber physiological age. In a series of 9 field trials with Home Guard and Maris Bard the physiological age beyond which total numbers of tubers decreased varied from 208 to 864 day-degrees above 4°C (O'Brien et al. 1983).

Plant Size and Tuber Bulking Rate

Madec and Perennec (1962) observed that plants from physiologically old seed grew faster than those from young seed. Storage of King Edward seed tubers at 2-3°C (i.e. physiologically young seed) resulted in plants with larger canopies than seed

tubers stored at higher temperatures (Wurr, 1978c). Allen et al. (1979) recorded that physiologically old seed tubers of Home Guard produced small plants with low tuber bulking rates. These plants exhibited a determinate growth form with fewer stems and leaves per stem. Physiologically ageing Maris Bard and Home Guard seed tubers produced plants with smaller peak leaf areas and earlier senescence (O'Brien et al. 1983). Scott and Wilcockson (1978) showed that there is a linear relationship between the amount of light intercepted by the potato plant's canopy and dry matter production. Bean and Allen (1980) suggest that the effect of physiological age on growth and yield can be explained by variation in the amount of light intercepted during the growth period.

Water Requirement

Advancing crop emergence and foliage development by pre-sprouting seed tubers means that the crop requires a greater quantity of water earlier in the growing season. If an early drought occurred the plants with the largest canopies would be most stressed (Lunden, 1944). O'Brien (1979) has demonstrated that the roots of plants from physiologically old seed tubers stop growing shortly after emergence. Plants grown from physiologically old seed tubers may therefore be vulnerable to shortages of water and nutrients.

Senescence

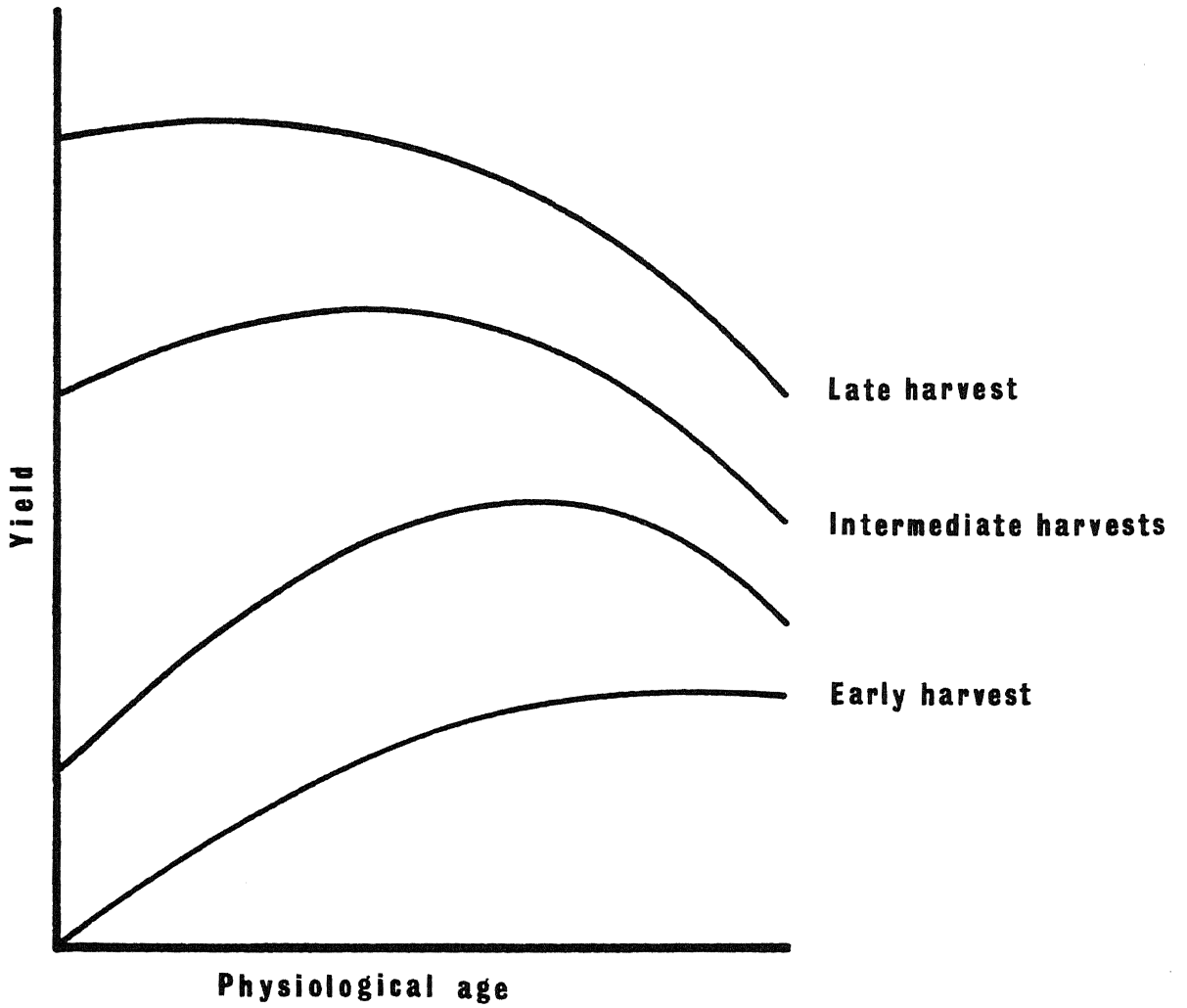
Headford (1962) and Headford and Ingersent (1962) recommended that maincrop potatoes be grown from physiologically young seed because old seed produced too little foliage which matured too early. These observations were confirmed by O'Brien et al. (1983) who found that physiologically old seed of Maris Bard and Home Guard produced the smallest peak leaf area and senesced earliest.

2.3.3 Physiological Age and Tuber Yield

Physiologically old seed tubers produce plants which emerge earlier, grow faster and initiate tubers earlier than plants grown from physiologically young seed tubers. This advancement of the vegetative growth period means that the plant can benefit from the larger water supply and higher light intensity found earlier in the growing season (O'Brien et al. 1983). It follows that, if harvested early, the yield from physiologically old seed tubers would be greater than from physiologically young seed tubers. This occurs for most of the achievable range of physiological ages in all maturity classes of potato and is supported by the findings of most researchers (Bus and Schepers, 1978; Perennec and Madec, 1980; Bean and Allen, 1980; O'Brien et al. 1983; O'Brien et al. 1986).

O'Brien et al. (1983) suggest that the effect of physiological age on yield changes progressively from early to late harvests. The general relationship is shown in Fig. 5. As the plant grows the effect of physiological age is determined by the balance between the positive effects (earlier canopy formation in growing conditions which are generally more favourable) and the negative effects (smaller maximum leaf area, greater susceptibility to water stress and earlier senescence). In early cultivars the effect of physiological ageing is large and is maximised in years when growing conditions are poor. Between 800 and 1000 day-degrees above 4 °C are required to maximise yields in Home Guard and Maris Bard (O'Brien et al. 1983). Early potato growers in Jersey are advised to condition seed tubers of the cultivar Jersey Royal to 700-900 day-degrees above 4 °C. Trials have shown that in many cases total yields were increased by ageing to between 700 and 900 day-degrees above 4 °C but in every trial the older seed tubers produced a greater ware

Fig.5 Suggested relationships between tuber yield and seed tuber physiological age for harvests throught the growing season (Adapted from O'Brien et al. 1983)



yield making physiological ageing economically viable (Anon. 1988a).

The effects of seed tuber physiological age on the yield of maincrop cultivars are more variable. Kawakami (1952), Iritani (1968) and Reust (1982) found that yields from physiologically old seed tubers were lower than those from physiologically young seed tubers. Other workers have noted little effect of seed tuber physiological age on yield (Fischnich and Krug, 1963; Bus and Schepers, 1978). The consensus of opinion is that physiologically ageing seed tubers has little effect on the yield of maincrop cultivars.

It is probable that for every cultivar and harvest date there is an optimum seed tuber physiological age which will maximise light interception and yield within the constraints of the length of the growing season (Allen and Scott, 1980).

2.4 TEMPERATURE AND THE DEVELOPMENT OF PCN

2.4.1 Introduction

While soil temperature influences all soil inhabiting nematodes, it is the key feature in the development and activity of endoparasitic species (Jones, 1983). Numerous reports have discussed how temperature affects hatching and the development of PCN with data from *in vitro* tests, glasshouse experiments and field trials. Field trial data exists for a range of soil temperatures from Scandinavia to Australia.

United Kingdom

Fenwick (1951) observed that temperatures greater than 21°C were unfavourable for the development of *Heterodera rostochiensis*. At 31°C development and multiplication were negligible as a result of slowed nematode development combined with a small decrease in juvenile (J2) invasion rates.

Jones and Parrott (1969) proposed that 4.4°C (soil

temperature at 10cm depth) was an appropriate basal temperature for the development of Heterodera rostochiensis in field plots at Woburn, Bedfordshire. They suggested that nematode development was controlled by the rate of heat accumulation rather than by soil temperature per se. Using data from Evans (1968) they calculated that invasion of roots by JJ2 began after approximately 300 day-degrees had been accumulated after the crop had been planted; most juveniles had developed into young adults after 1000 day-degrees (c. 85 days after planting). Males and females were abundant after 1500 day-degrees (c. 106 days after planting) and 3-4 weeks later (127-134 days after the crop had been planted) females that were mature and full of eggs were visible on the roots.

In hatching tests at 25°C, McKenna and Winslow (1972) noted that pathotype A Heterodera rostochiensis always hatched faster than pathotypes B and E. Pathotypes A, B and E are equivalent to Ro1, Pa1 and Pa2/3 respectively in the current European pathotyping scheme (Stone et al. 1986). Although the test temperature was much higher than the 0-15°C range found in field soil during the experimental period, the authors considered that Ro1 exhibited greater "readiness" to hatch in spring than Pa1 or Pa2/3. This was confirmed in outdoor microplots where Ro1 developed faster than Pa1 and Pa2/3.

Ellenby and Smith (1975) found that populations of Heterodera rostochiensis pathotype A (Ro1) from Ayrshire and Newcastle both hatched well from cysts in vitro at 23°C. The Ayrshire population was from a field where early potatoes had been grown continuously for 80 years. At 10°C a greater proportion of Ayrshire than Newcastle cysts hatched, suggesting that the Ayrshire population had become adapted to cultural practices in the field. Repeated early potato harvests had selected those nematodes which were able to hatch and develop at low

temperatures, enabling them to complete their life cycle before the potatoes were lifted. This "adaptation" was later confirmed by Hominick (1979).

Hatching tests *in vitro* at 10-30°C with 12 populations of *G. rostochiensis* and 14 of *G. pallida* indicated that *G. pallida* hatches less freely than *G. rostochiensis* at higher temperatures. Parrott and Berry (1976) concluded that *G. pallida* is adapted to slightly lower temperatures than *G. rostochiensis*. The optimum temperatures for hatching were 19-22°C and 16°C for *G. rostochiensis* and *G. pallida* respectively. Berry, Stone, Parrott and Al-Sakaff (1978) also found the optimum temperature for the hatching of *G. rostochiensis* to be higher than for *G. pallida*.

Webley and Jones (1981) used the correlation between mean daily air temperatures and mean soil temperatures to calculate accumulated temperatures, in day-degrees, above an "assumed" basal temperature for development of 10°C for PCN infested microplots in South Wales during 1971-1978. The relationship between accumulated temperatures and post harvest population densities was fitted by a maximum likelihood programme (Ross, 1980) to a negative exponential curve. This analysis indicated that females of *G. pallida* require fewer day-degrees to develop and produce eggs than females of *G. rostochiensis*.

Europe

In France, *in vitro* tests showed the basal temperature for development of *G. pallida* and *G. rostochiensis* to be 3.9 and 6.2°C respectively. *Globodera pallida* competed successfully with *G. rostochiensis* at 9.5°C but not at 24°C (Mugniery, 1978). The further study of three populations of *G. pallida* and two of *G. rostochiensis* revealed development thresholds of 4.5, 5.3, 6.8, 6.3 and 5.9°C respectively (Langeslag, Mugniery and Fayet, 1982).

In Cyprus, invasion by JJ2 of *G. rostochiensis* occurred

approximately 34 and 44 days after planting in autumn and spring planted potato crops when 10cm soil temperatures were 22 and 14.5℃ respectively. To develop from JJ2 to embryonated eggs took 56 and 63 days for autumn and spring crops, and with an assumed basal temperature for development of 10C this represented 529 and 416 day-degrees. A second generation was initiated on the spring crop but only one generation per year was completed (Philis, 1980).

Greco et al. (1980) used the same "assumed" basal temperature for development of G. rostochiensis in Italy, where two generations can be completed in one year. JJ2 were able to invade potato roots when the mean soil temperature reached 10℃. Development from JJ2 to adult female required 168 or 200 day-degrees, while JJ2 to cysts (with embryonated eggs) needed 450 or 342 day-degrees for the first and second generations respectively.

North America

Chitwood and Buhrer (1946) found that very limited hatching of Heterodera rostochiensis occurred at mean soil temperatures below 11.1℃. The entire life cycle (from embryonated egg to embryonated egg) took between 38 and 48 days at Long Island, New York. Ferris (1957) also observed that soil temperature had a marked effect on the life cycle of H. rostochiensis. Root invasion was lower at 29.4℃ than 18.3 or 23.9℃ and nematode development within the roots of plants previously grown at 18.3℃ ceased when the soil temperature was raised to 29.4℃. Soil temperatures of 29.4℃ for longer than 5 days caused nematodes within the roots to degenerate.

In field plots in Newfoundland, JJ2 of H. rostochiensis were not observed until weekly mean soil temperatures at 7.6cm depth were 15.2, 15.2, 16.7 and 14℃ for the years 1963-1966. One generation was observed per year, the life cycle being completed

in 50-58 days (Morris, 1971).

Scandinavia

Hansen and Jakobsen (1985) and Magnussun (1986), working with Danish and Swedish populations of G. rostochiensis, used a basal temperature for development of 6°C. The Danish population required 440 day-degrees for the development of females, which corresponded well with the Swedish population which needed 400-500 day-degrees for the development of juveniles into young adults.

Hatching of G. rostochiensis in Finnish soil was first observed when the 10cm soil temperature reached 4-5°C, and was followed by invasion when the temperature reached 10°C. The complete life-cycle required 765±34 day-degrees above an assumed base for development of 4.4°C (Tiilikkala, 1987).

New Zealand and Australia

Foot (1978) reported that, in New Zealand, G. pallida Pa2 would develop at 8-22°C, Pa3 at 9-23°C and G. rostochiensis at the slightly higher range of 10-25°C; for all populations the optimum was 15-20°C. Below 13-14°C, G. pallida developed more rapidly than G. rostochiensis but at 15-20°C the situation was reversed. The generation interval at 23°C was 7-8 weeks and this increased to 14-15 weeks at 7.8°C.

In Western Australia, Stanton and Sartori (in press) observed that G. rostochiensis took 49 days to complete its life cycle. Optimum temperatures for hatching (in picrolonic acid) and egg development were 22°C and 15°C respectively. Second generation JJ2 were observed but they did not develop any further.

2.4.2 Use of Temperature and Development Data

Detailed information regarding the effect of temperature on the rate of nematode development is only available for a few species (Tyler, 1933; Jones, 1977; Greet, 1978). Complete rate/temperature curves are desirable for all major species of

plant parasitic nematodes (Jones, 1983). If appropriate data is available, descriptic curves can be fitted and upper and lower temperature thresholds estimated using the method of maximum likelihood as described by Ross (1980). This method portrays developmental rates at low temperatures, and temperatures beyond the optimum, more accurately than using accumulated temperatures as an expression of nematode development.

For field trials, meteorological data is used in the calculation of accumulated temperatures but there are several problems associated with this that have been discussed by Baker (1980). However, the soil environment is reasonably stable and so cyst nematode development can be accurately modelled using accumulated temperature above an assumed threshold temperature (Jones, 1983) and this has been done for the sugar beet cyst nematode Heterodera schachtii (Jones, 1975).

Detailed temperature and development information for PCN would allow the mean development stage of the nematodes to be predicted at any time during the growth of the potato crop. This would allow accurate phenological control of PCN in early potato crops in the U.K. Phenological control relies on harvesting the potato crop before the nematode can form mature cysts and complete its life cycle. It has been practised in Ayrshire, Scotland, for many years (Hominick, 1979) as well as in Belgium (van den Brande and d'Herde, 1964) and Cyprus (Jones, 1976). In Cyprus, where three potato crops can be grown in a year, trials have shown that G. rostochiensis could be phenologically controlled in the spring and winter growing seasons (Philis, 1986).

Webley and Jones (1981) conclude that harvesting an early potato crop a fixed number of days after planting (less than 100 in the U.K.) would give satisfactory PCN control in most years but harvesting on the basis of accumulated day-degrees above a

basal temperature for development would be more reliable, especially in years with extreme weather patterns.

One full generation of PCN is completed during a growing season in the U.K. although there is evidence that a second generation is initiated in crops growing late into the autumn (Evans, 1968). It has been predicted that by the year 2050 carbon dioxide concentrations in the atmosphere will have doubled from pre-industrial revolution levels to 540 ppm. As a result of the "greenhouse" effect, models predict that this will probably cause a mean temperature rise of 3 °C (± 1.5 °C) (Anon., 1988b). Detailed temperature and development information could be used to predict how this temperature increase would affect the nematode's life cycle. In particular, the possibility of a second generation would have important implications for the management of PCN populations in the future.

2.5 IMMUNOLOGY AND THE ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

2.5.1 Introduction to Immunology

Immunology is the study of the immune responses elicited in animals in their fight to defend themselves against foreign (pathogenic) organisms. While the antibody mediated humoral and cell mediated responses of the immune system both involve cells of the lympho-reticular system, it is the antibodies involved in humoral immunity that are used in immunochemical techniques.

An antigen may be a foreign organism or compound and, upon entry into animal tissue, it stimulates lymphocytes to divide and differentiate. Eventually plasma cells are formed which secrete antibodies specific to the causal antigen. Antibody molecules contain sites which can bind tightly to complementary regions (epitopes) on the original antigen causing the precipitation of a macromolecule, neutralisation of a toxin or death of a micro-organism.

Antibodies are protein immunoglobulins (Ig) of which there are five classes:- IgG, IgM, IgA, IgD, and IgE. IgG is used in immunochemical techniques and constitutes approximately 80% of serum immunoglobulins. An immunoglobulin molecule consists of two identical "heavy" and two identical "light" polypeptide chains linked by disulphide bridges, causing a compact globular structure (Wilson and Goulding, 1988).

2.5.2 Polyclonal Antisera

A polyclonal antiserum may be raised to a single antigen (though in practice many antigens may be involved) but even then it contains a number of antibodies to the antigen from different plasma cell clones; therefore all antisera are polyclonal.

Antisera Production

Antibodies used in immunochemistry are mostly raised by the injection of a suspension or solution of an antigen into a rabbit. Inoculation of a strongly antigenic saline solution results in the production of specific IgM antibodies as a part of the primary humoral immune response. Antibodies can be detected in the serum after approximately 10 days, reach a maximum 15-20 days after inoculation and then decline in concentration over a period of weeks. A further antigen injection produces a more rapid secondary response with increased antibody levels detectable after 3 days and maximum concentrations occurring 10 days after the second injection. The secondary response produces similar amounts of IgM antibody to the primary response but three to ten times the quantity of IgG antibody. Subsequent antigen injections at intervals of 2 weeks produce a hyperimmunised rabbit containing 1-5mg of specific IgG antibody per ml of serum.

To obtain antisera with readily detectable levels of specific antibodies (i.e. with sufficient titre) from weakly antigenic inoculants, the standard procedure can be modified in two ways. Firstly, exposure of the immune system to the antigen

may be lengthened by repeated inoculation or by intramuscular, subcutaneous or intradermal inoculation of precipitated antigen which releases antigen over several weeks. Secondly, an adjuvant such as "Freund's complete adjuvant" may be used to enhance the antigenicity of the compound. This adjuvant contains white mineral oil, emulsifier and heat-killed Mycobacterium tuberculosis (Bomford, 1984). By inoculating an emulsion (comprising equal volumes of antigen solution and adjuvant) into three different sites within the rabbit, the number of lymph nodes stimulated is increased. The dead tubercle bacilli further enhance the immune response to the antigen. Final inoculations before bleeding use "Freund's incomplete adjuvant" which lacks the tubercle bacilli and only stimulates the humoral immune response.

Between 5 and 50ml of blood is removed from the immunised rabbit and is allowed to clot at 37℃ for an hour. The detached clot is left at 4℃ to contract and exude 2-25ml of serum. Heat treatment at 56℃ for 45 minutes inactivates proteases and enables the safe storage of small aliquots of serum at -20℃.

Large antigens such as proteins stimulate the production of a multitude of different immunoglobulins, each reacting with a slightly different epitope on the antigen's surface. The relative proportion of each immunoglobulin in a polyclonal serum varies according to the method and frequency of antigen inoculation, the nature of the antigen and the adjuvant used. Standardisation of antigen specific polyclonal antisera is extremely difficult and it is impossible to obtain a homogeneous population of a single molecular species of immunoglobulin molecule from polyclonal antisera(Wilson and Goulding, 1988). Modern cell biological techniques have, however, enabled the production of monoclonal antibodies.

2.5.3 Monoclonal Antibodies

The technology to produce monoclonal antibodies was first developed by Kohler and Milstein in 1975. The fusion of a plasma cell with a transformed myeloma cell line forms a monoclonal antibody-secreting hybridoma. As a plasma cell produces a specific antibody a cloned hybridoma line can produce a limitless quantity of antibody which will only recognise a single epitope. Impure antigens can also be used to raise a specific antibody to an epitope of particular interest.

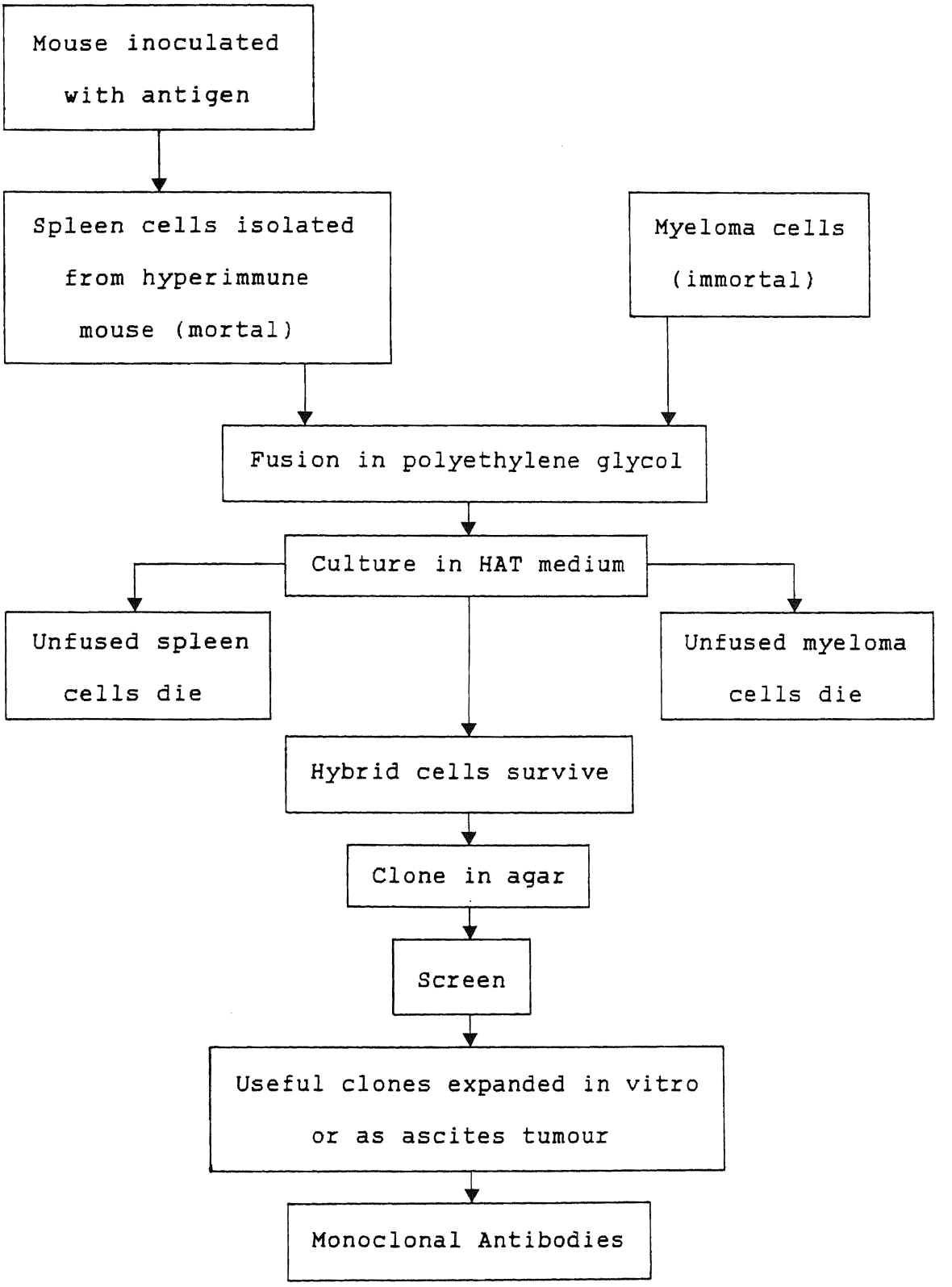
Production of Monoclonal Antibodies

Monoclonal antibodies for research are usually raised in rodents (Fig. 6) because efficiently fusing myeloma cell lines which form stable hybridomas are readily available.

An intraperitoneal inoculation of between 10 and 100µg of adjuvated antigen into the rodent is repeated several times until antibodies that bind to the antigen can be detected in the blood. When this occurs hybridomas can then be produced from the boosted and rested responder rodent. The spleen is removed from the responder rodent 3 days after the last antigen injection and the splenocytes are extracted. Approximately one myeloma cell per 10 splenocytes are mixed and fused by treatment with 50% polyethylene glycol (PEG) in physiological saline. Cells are transferred to multiwell plates containing a growth medium, for example, Dulbecco's modified Eagle's medium (DNEM) supplemented with 10-20% animal serum. Hybridomas can also be maintained in serum-free media but these are relatively expensive (Barnes, Sirbasku and Sato, 1984).

Not all cells fuse and so the mixture contains cells of the transformed myeloma line and mortal splenocytes as well as the hybrid cells. If left the myeloma cells would quickly overgrow the hybridoma cells and so the Littlefield selection system is adopted to kill the myeloma cells (Littlefield, 1964). There are

FIG. 6 Monoclonal antibody production in mice



two pathways of nucleotide biosynthesis; the usual synthetic route which can be blocked by aminopterin and a backup mechanism involving phosphoribosyl transfer catalysed by hypoxanthine guanine phosphoribosyl transferase (HGPRT) to reuse existing bases. In the presence of aminopterin, myeloma cells (which have been selected for the loss of the enzyme HGPRT) cannot synthesise purine nucleotides but hybridomas (having HGPRT activity from the splenocyte) are able to and can therefore survive. The selective HAT medium contains hypoxanthine (H), aminopterin (A) and thymidine (T).

After a week to ten days colonies of hybridomas are growing visibly in the HAT medium. Hundreds of different colonies are generated from the fusion of a single mouse spleen and either solid phase radiobinding or enzyme linked immunoassays are used to screen and discard undesirable clones. Reactive hybridomas are cloned from single cells to ensure that only a single antibody specificity is represented. Cloning is repeated several times and the hybridomas can be stored in liquid nitrogen and removed when required to produce a continuous supply of a specific antibody (Wood, 1987).

If large quantities of monoclonal antibodies are required then ascitic (fluid peritoneal) tumours can easily be induced in rats and mice. The myeloma lines used have been derived from strain LOU rats and BALB/c mice and to prevent immunological rejection hybridomas grown as ascitic tumours must be produced in the same strain.

Because of their reproducible qualities, monoclonal antibodies are ideal reagents for immunoassays. One very useful immunoassay is the enzyme linked immunosorbent assay.

2.5.4 The Enzyme Linked Immunosorbent Assay

The enzyme linked immunosorbent assay (ELISA) has several variations which provide sensitive methods for detecting both

antigens and antibodies (Voller, Bidwell and Bartlett, 1979). This technique was first reported by Engvall and Perlman (1971) and van Weeman and Schuurs (1971).

An antigen (or antibody) is immobilised on a solid surface which is usually a 96-well plastic microtitre plate. The corresponding antibody (or antigen) used to detect the immobilised molecule is linked to an enzyme which forms a conjugate with both enzymatic and immunological reactivity. After incubation of the antibody with the immobilised antigen and several washes to remove unreacted reagents, a substrate is added which is converted by the linked enzyme into a highly chromatic product. Colour density is read using a spectrophotometer and is a measure of the amount of enzyme in the solution which is equivalent to the quantity of antigen-antibody complex.

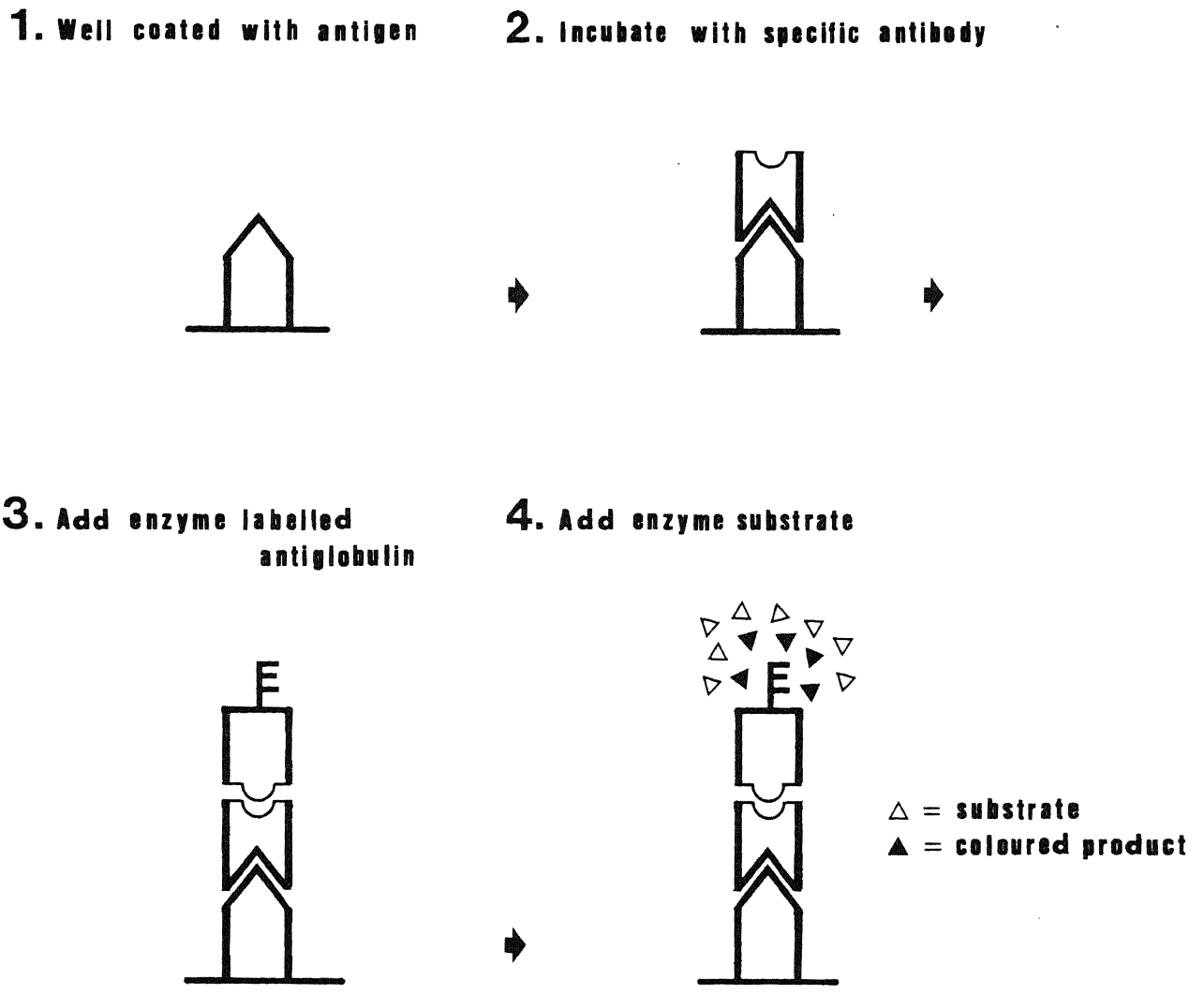
ELISA Methods

The many variations of ELISA can be divided into two basic types: non-competitive and competitive assays, illustrated in Figures 7 and 8 by the indirect and competitive methods used to detect antigens (or antibodies) in samples. Although an ELISA can take between 1 and 3 days to complete, most of the time is taken up by incubation periods.

Adsorption of the Antigen or Antibody onto the Solid Support

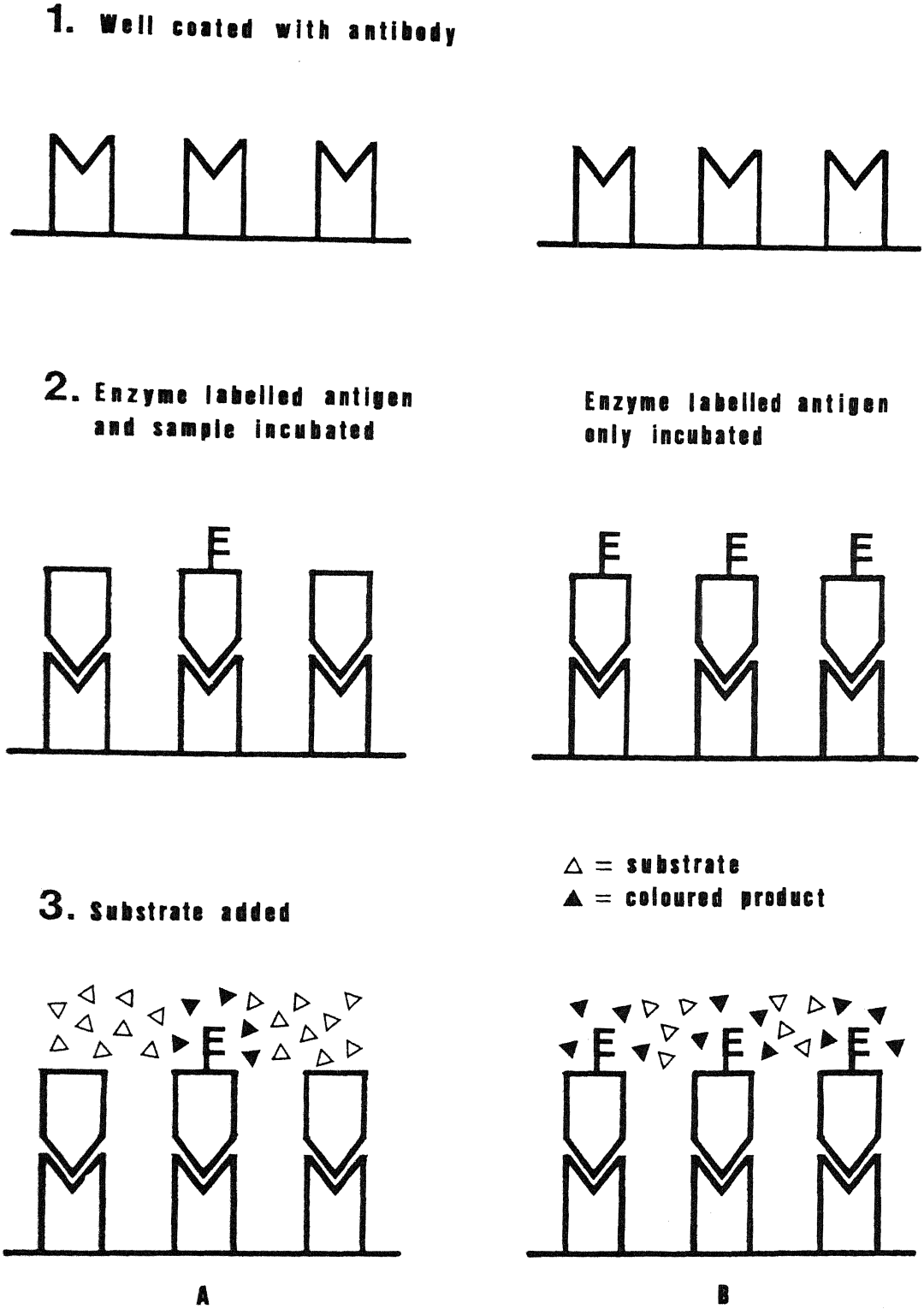
Adsorption of the antigen or antibody onto the surface of the microtitre plate is a passive process occurring as a result of hydrophobic interactions. Optimal conditions for plate coating need to be determined for each antigen or antibody used. For most proteins a concentration of 1-10µg/ml in carbonate or bicarbonate buffer at pH 9.8 for 2-3 hours is adequate. However, for convenience coating overnight at 4°C is widely practised. Approximately 30% of the plate coating is lost by elution during the course of the assay and this percentage may be increased at high antigen or antibody concentrations. Coating variability is

Fig.7 The indirect ELISA for the detection of antigen



N.B. The optical density of the coloured product is proportional to the quantity of enzyme labelled antibody which is in turn a direct measure of the amount of antibody and antigen.

Fig.8 The competitive ELISA for the detection of antigen



N.B. The greater the quantity of sample antigen in A the less coloured product will be produced. The difference between colour densities of A and B is a measure of the quantity of antigen in the sample.

an important factor determining the precision of an ELISA. It is possible to store dried, airtight, coated plates at 4℃ for over a year. Non-specific adsorption, for example, of antibody directly onto a plate that has been incompletely coated with antigen, can be overcome by "blocking" spare binding sites with a non cross reacting protein such as casein or albumin. Non-ionic detergents such as "Tween 20" can be used in diluents and washing buffers as a precaution against non-specific adsorption.

Enzyme Labels

The two most commonly used enzyme labels for ELISA are horseradish peroxidase (HRP) and alkaline phosphatase (AP). The substrate for HRP is hydrogen peroxide where its cleavage is coupled to the oxidation of the hydrogen donor (chromogen); for example, tetramethylbenzidine (TMB) is oxidised to form a dark blue product. Colourless p-nitrophenylphosphate is hydrolysed to yellow p-nitrophenol by AP. Enzyme linked antibodies such as anti-rat IgG alkaline phosphatase are commercially available for use in ELISA tests.

Sensitivity

An enzyme linked antibody-antigen complex can be detected at concentrations as low as 1fmol of antigen per 200µl volume. In practice, the sensitivity of ELISA is limited by the affinity of the antibody for the antigen which is reduced in some procedures, for example by the partial denaturation of the antigen or antibody when it is immobilised on the plate surface. ELISA has a sensitivity comparable to radioimmunoassay (RIA) shown by the work of Wolters, Kuypers, Kacaki and Schuurs (1977) who reported that ELISA could detect hepatitis B antigen at levels of 5-10ng/ml of blood, which is very similar to that detectable by RIA. When compared with haemagglutination, ELISA was up to 30, 160 and 200 times more sensitive at detecting antibodies to

cytomegalovirus (Castellano, Hazzard, Madden and Sever, 1977), Epstein-Barr virus (Wallen, Mattson and Levine, 1977) and herpes simplex virus (Gilman and Docherty, 1977) respectively.

2.5.5 ELISA in Plant Pathology

The adaptation of the medical immunodiagnostic procedure ELISA for use in plant pathology was first described in the late 1970's for the detection of viruses (Voller et al., 1976; Clark, and Adams, 1977).

The advantages that ELISA had over other methods were:-

(i) detection of pathogens at lower concentrations than was possible by classical immunoprecipitation methods.

(ii) the ability of ELISA to detect antigens of different size and morphology meant that the same basic protocol could be used for a range of plant pathogens.

(iii) the potential of ELISA to provide quantitative data for advisory or research purposes.

(Clark, 1981)

ELISA has been successfully used in the identification of viral diseases in a range of crops, for example potatoes (Tamada and Harrison, 1980; De Bokx, Piron and Maat, 1980), fruit (Flegg and Clark, 1979; Bar-Joseph, Sacks and Garnsey, 1978; Clark et al., 1976) and hops (Barbara, Clark and Thresh, 1980; Thresh et al., 1977). Bacteria and fungi have also been assayed by ELISA (Cother and Vruggink, 1980; Kishnevsky and Bar-Joseph, 1978; Casper and Mendgen, 1979).

2.5.6 Immunology and Plant Parasitic Nematodes

Immunological studies of plant parasitic nematodes have been confined mainly to root knot and cyst nematodes (Fox and Atkinson, 1986). Due to the small size of nematodes, whole body homogenates have been used rather than specific secretions or tissue types.

Bird (1964) found that antibodies raised in rabbits to

Meloidogyne javanica reacted with the surface of the nematode's cuticle or to stylet secretions. Webster and Hooper (1968) raised antisera to various species of Ditylenchus and Heterodera. Using gel diffusion they obtained reactions between species within each genus but no cross reactivity between genera. They found no differences between G. rostochiensis and G. pallida but were able to distinguish PCN from Heterodera species.

Fox and Atkinson (1985) carried out a detailed serological study using crossed immunoelectrophoresis to examine populations of G. pallida and G. rostochiensis. An antiserum raised to G. rostochiensis reacted with antigens from four Heterodera species but produced less precipitation lines than were observed with G. rostochiensis and G. pallida. Investigations revealed the PCN antigens to be hydrophilic with acidic isoelectric focusing points; lipoproteins, peptidase and acid phosphatase were all detected in the immunoelectrophoretic arcs. Crossed immunoelectrophoresis of field populations and reference PCN pathotypes revealed several species-specific arcs in the reference populations. They concluded that the purification of species-discriminating antigens, for example an immunologically distinct protein, could lead to the development of a simple diagnostic immunological test such as ELISA.

2.5.7 ELISA to Detect Plant Parasitic Nematodes

If monoclonal antibodies or polyclonal antisera could be raised to a species of plant parasitic nematode or even pathotypes within a species then ELISA could be used to detect the presence of a species or pathotype in a root or soil sample. This would be very useful for advisory work and chemical and resistance screening programmes. ELISA tests for Meloidogyne spp. would remove the need for time consuming visual determination of root knot indices; for PCN soil population densities could be assessed without the laborious processes of sieving, flotation

and two separate microscope counts required at present.

Robinson (1989) has raised monoclonal antibodies and polyclonal antisera to Meloidogyne spp. and used ELISA techniques to detect and quantify the nematode in root and soil samples. Both assays have been designed for future development into diagnostic kits. Monoclonal antibodies and polyclonal antisera have been raised to G. pallida and G. rostochiensis (M. Robinson, pers. comm.) and it is possible that a quantitative PCN assay could be developed.

CHAPTER 3

FIELD TRIALS 1 AND 2

CHAPTER 3 FIELD TRIALS 1 AND 2

3.1 INTRODUCTION

3.1.1 Rosedene Farm

The field trials in 1987 and 1988 were carried out at Rosedene Farm, Methwold Hythe, Norfolk. This 295ha arable farm owned by Greens of Soham Ltd has fertile organic fenland soil. Potatoes are usually grown in a 3 year rotation with onions and carrots but some fields have grown 2-4 consecutive potato crops, which has resulted in a rapid increase in PCN population densities in the soil. In these fields, PCN are reducing yields to the extent that potatoes are no longer a profitable crop (W. Smith, pers. comm.). *Globodera pallida* is most abundant, occurring in pure populations in some fields and as mixed populations with *G. rostochiensis* in others (Whitehead and Westerdijk, 1987).

3.1.2 Site Selection

In 1986, the Agricultural Development and Advisory Service (ADAS) took soil samples and estimated PCN population densities for every field on the farm. All fields that were scheduled to grow potatoes in 1987 were potential trial sites and their mean estimated PCN population densities are shown in Table 6. Field No. 1 was chosen as the trial site because of its relatively low mean PCN population density and its close proximity to farm buildings and access roads (Plate 2).

3.1.3 Preliminary Soil Sampling

In March, 1987 Field No. 1 was marked out into 48 squares of 25 X 25m. Using a 25 X 250mm "cheese corer" soil sampler, 20 cores were removed from each square in a "W" pattern, bulked into a hessian bag and air dried at 20°C for 4 weeks.

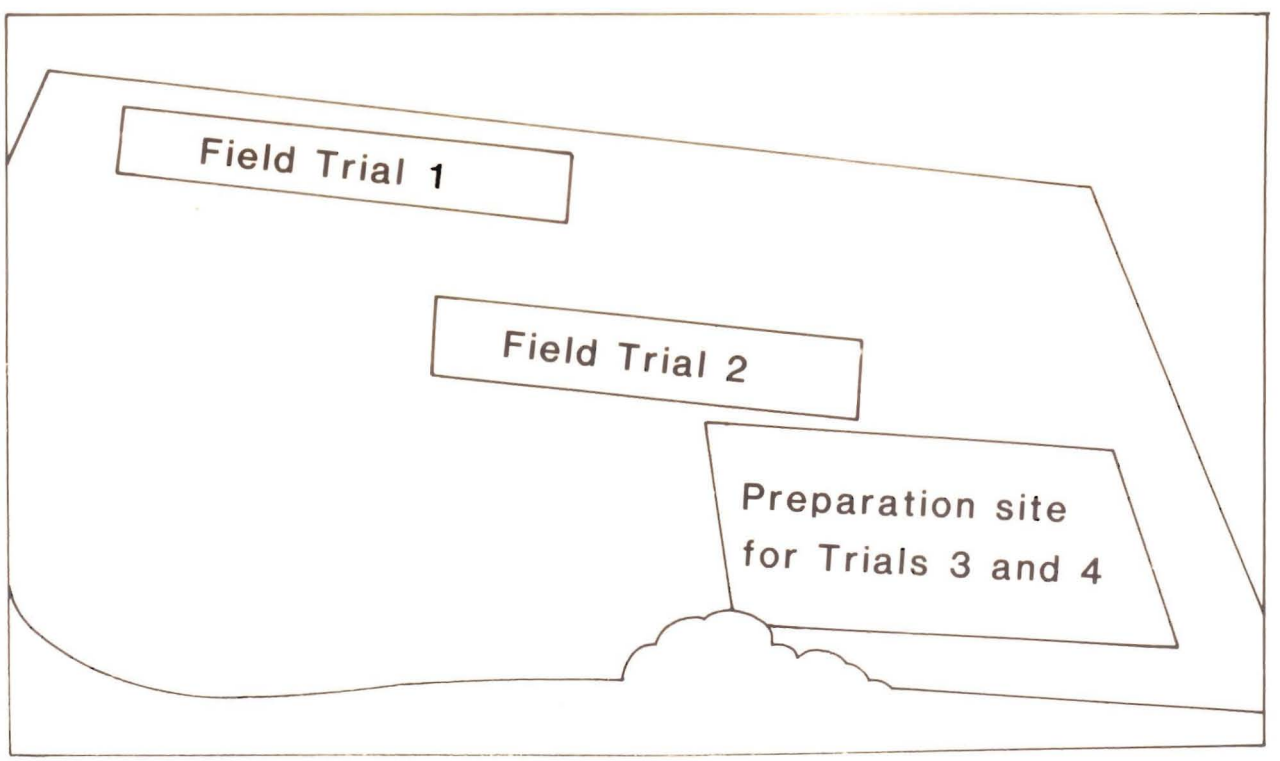
3.1.4 PCN Population Density Estimation

The air dried soil was sieved and thoroughly mixed before

TABLE 6 PCN population densities at Rosedene Farm (1986)

Field	Area(ha)	Full cysts/100g soil
1	4.48	50
2	--	84
3	3.93	114
19	6.19	83
20	7.59	170
21	8.15	63
22	9.72	110
23	10.49	113
24	9.94	50

Plate 2 Aerial photograph of Field 1 (7 July, 1987)



the cysts were extracted from a 100g sub sample by elutriation using a Fenwick Can (Fenwick, 1940). The cysts were then counted and their mean egg content estimated by standard techniques (Shepherd, 1986). The same method of PCN population density estimation was used for all field trials. PCN population densities estimated for Field No. 1, expressed as eggs/g soil are shown in Fig. 9. Iso-electric focusing (Fleming and Marks, 1983) using crushed cysts on polyacrylamide gel indicated that Field 1 had a pure population of G. pallida with no G. rostochiensis detectable (Plate 3).

3.2 FIELD TRIAL 1

The effect of seed tuber physiological age and nematicide treatment on the growth and yield of maincrop cultivars Pentland Dell and Maris Piper grown in plots infested with G. pallida.

3.2.1 Experimental Design

The trial required an area of land with a uniform initial nematode population density and was sited within grids 45-47 of the preliminary survey of Field No. 1 which had a mean estimated Pi of 282 eggs/g of soil (Fig. 9).

Seed tubers of Pentland Dell and Maris Piper were conditioned to 3 physiological ages (0, 200 and 400 day-degrees above 4C) and planted in plots split for nematicide treatment.

The trial consisted of 3 blocks each containing 12 split plots (Fig. 10) where a split plot contained 2 inner harvest rows (for final yield assessment), 2 sample rows (for growth analysis) and 2 outer guard rows. There were 20 plants in a row guarded at each end by 2 Desiree plants (Fig. 11). Rows were 86cm wide with seed tubers planted at 30cm spacing which was common to all field trials.

FIG. 9 Preliminary survey of PCN population density in Field 1

1 237	2 107	3 68	4 74	5 66	6 41	7 27	
8 58	9 69	10 238	11 239	12 217	13 109	14 68	15 124
16 201	17 131	18 426	19 331	20 444	21 262	22 562	23 190
24 134	25 102	26 166	27 213	28 357	29 153	30 212	31 215
32 47	33 51	34 72	35 75	36 142	37 240	38 311	39 247
40 144	41 154	42 122	43 136	44 144	45 259	46 314	47 273

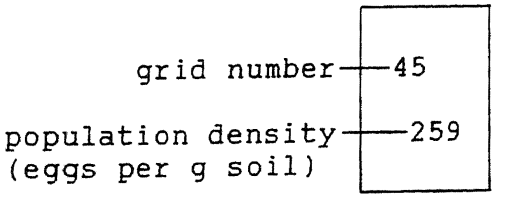
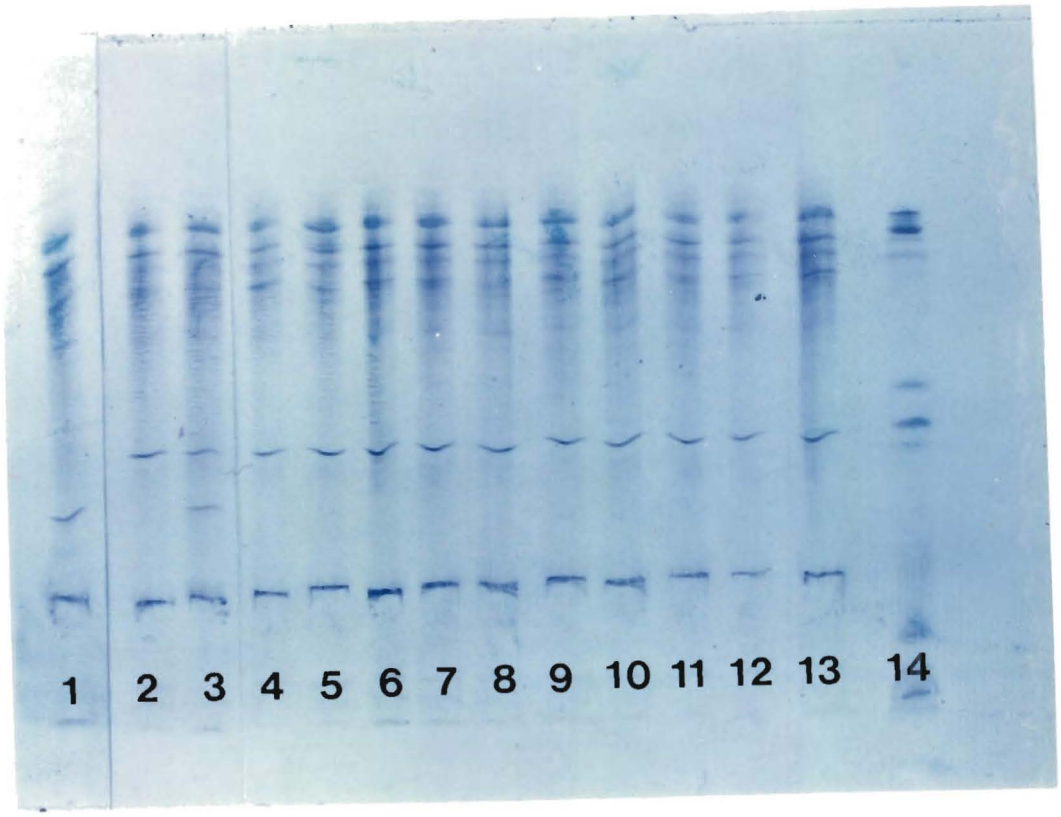


Plate 3 Identification of G. pallida in Field 1 using iso-electric focusing on polyacrylamide gel



1 = G. rostochiensis
2 = G. pallida
3 = Mixture of 1 and 2
4-13 = Field 1 samples
14 = Marker protein

} Pure stocks from Rothamsted

Pa = G. pallida band
Ro = G. rostochiensis band

FIG. 10 Field Trial 1 : Experimental design

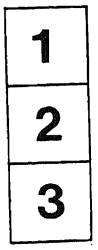
One block (all three blocks identical):-

Pentland Dell 400 d.d. +N -N	Maris Piper 0 d.d. -N +N
Maris Piper 200 d.d. -N +N	Pentland Dell 0 d.d. +N -N
Maris Piper 400 d.d. +N -N	Pentland Dell 200 d.d. -N +N

+N = 4.4 kg/ha aldicarb

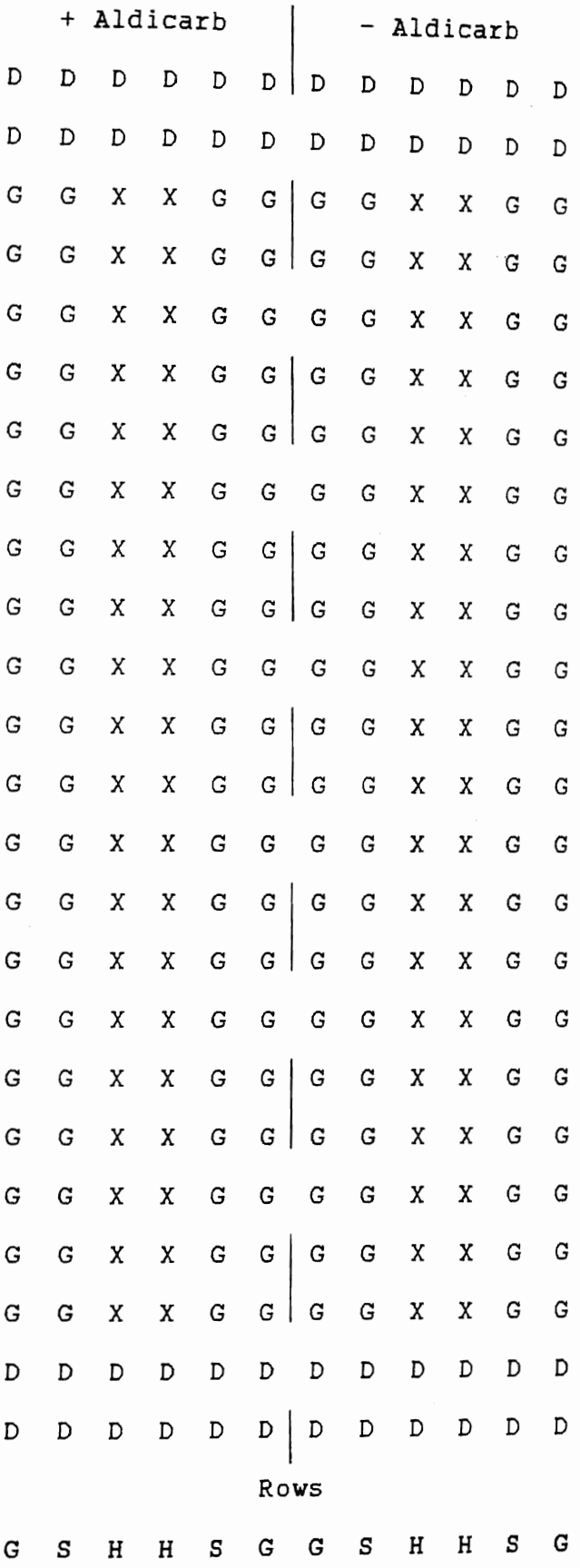
-N = no aldicarb

d.d. = day-degrees



Block layout

FIG. 11 Field Trial 1 : Split plot design



H = Harvest row
G = Guard row
S = Sample row
X = Harvest plant
D = Desiree guard plant
G = Guard plant (same seed as harvest plants)

3.2.2 Methods

Physiological Ageing of Seed Tubers

Sufficient healthy 30-40g seed tubers were selected from Scottish SE2 grade seed to allow for 15% wastage during storage (Plate 4).

A single layer of seed tubers were placed in wooden trays in an illuminated chitting store kept at 12°C until 90% of the tubers had sprouts which were 3mm in length i.e. they had broken dormancy. Tubers that did not require physiological ageing (the 0 day-degree treatment) were then transferred to a 3°C store until removal for planting. The remaining tubers were retained at 12°C until they had accumulated 200 or 400 day-degrees above 4C before transfer to the 3°C store.

Nematicide Application

On May 20, aldicarb (Temik; Embetec) was broadcast onto plots at 4.4kg a.i./ha, using a hand propelled granule applicator, and was immediately rotavated into the top 20cm of soil. Rotavation was in the direction of the rows and 3m was left between plots to allow for nematicide drift.

Planting

On 2 and 3 June, seed tubers were planted by hand at a depth of approximately 5cm into fully formed ridges.

Weed and Disease Control

Metribuzin (Sencorex WG; Bayer) at 1.5 kg/ha was applied on June 10 as pre-emergence weed control. After emergence, plots were weeded by hand as required. Mancozeb and Metalaxyl (Fubol 75; Ciba Geigy; 67.5 and 7.5% a.i. respectively) was applied at 2kg/ha in response to ADAS blight warnings. On 13 July a small blight outbreak was found. This was confirmed on 15 July and a small amount of infected plant material was removed from the plots.

Plate 4 Seed-tuber preparation

Grading seed-tubers by weight

Graded seed-tubers ready for conditioning

Canopy Development

Percentage ground cover was measured weekly for every plot from 25 June to 8 September using a viewing frame (Evans, Parkinson and Trudgill, 1975; Evans, 1982b).

Growth Analysis

After percentage ground cover was measured, 2 whole plants were carefully removed from each plot, dissected and the following data recorded:-

1) The number of tubers with diameter of 10mm or more, their fresh weight and dry weight.

2) The number of nodes both above and below ground level.

3) The number of stems.

4) The fresh weight and dry weight of the stems and leaves.

5) The number of stolons, their fresh weight and dry weight.

6) The fresh weight and dry weight of the root system.

A 2g sub sample of each root system was preserved in formal-acetic alcohol (FAA; Hooper, 1986).

Soil Sampling

Pi and Pf were estimated from 30 cores of soil taken before planting and immediately after harvest (Section 3.1.4).

Harvesting

Harvest plants were lifted by hand, using a fork, between 28 September and 1 October. Potatoes from each plot were weighed and ware yield estimated by riddling over a 40mm mesh; tubers passing over the grid constituted ware yield.

Data Analysis

Data from all field trials were analysed using analysis of variance (ANOVA) on Genstat 5, Release 1.2 at Rothamsted Experimental Station.

3.2.3 Results and Discussion

Yield

Mean total, ware and percentage ware yield for each

treatment are summarised in Table 7. Tuber yields from this trial were low with a mean ware yield of 8.25 t/ha accounting for only 52.6% of the total yield of 12.14 t/ha. this suggests that tubers were small and immature at harvest.

The total and ware yields of Maris Piper were significantly greater than those of Pentland Dell ($P< 0.01$, Table 8) and as Pi's were not significantly different between cultivars (Appendix 1) this implies that Maris Piper is more tolerant of attack by G. pallida than Pentland Dell.

As the physiological ages of seed tubers were increased, both total and ware yield became greater (Table 9); 400 day-degree seed tubers had significantly greater yields than 0 day-degree seed tubers ($P< 0.05$) but not 200. Both Maris Piper and Pentland Dell produced larger yields from 400 than 0 day-degree seed tubers (Table 10 and Fig. 12) although these differences were not significant. When the ware yield of Pentland Dell is expressed as a percentage of that of Maris Piper (Table 11) this confirms that Maris Piper is more tolerant of attack by G. pallida than Pentland Dell. The yield of both cultivars is increased by physiologically ageing seed tubers but this effect is greater for the intolerant cultivar Pentland Dell (Table 11).

When all treatments are considered (Table 12), aldicarb application increased total and ware yields significantly when compared with untreated plots ($P< 0.05$ and 0.01 respectively). Total and ware yields of Maris Piper were significantly increased by aldicarb treatment ($P< 0.05$ and 0.01) but only the total yield of Pentland Dell was increased ($P< 0.05$, Table 13 and Fig. 13).

TABLE 7 Field Trial 1: Mean yields for all treatments

Cultivar	Day-degrees	Aldicarb	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
P. Dell	0	-	6.88	2.07	24.7
		+	3.68	0.31	6.6
	200	-	2.39	0.26	9.5
		+	10.26	4.83	46.9
	400	-	8.09	3.69	40.7
		+	11.44	6.44	54.5
M. Piper	0	-	10.56	6.52	52.0
		+	18.35	14.50	78.8
	200	-	14.29	10.65	68.9
		+	28.83	20.36	84.9
	400	-	16.88	14.36	85.0
		+	19.02	15.07	78.5
S.E.D.(2 d.f.)			2.042	2.119	9.30

TABLE 8 Field Trial 1: Cultivar mean yields

	Cultivar		
	Pentland Dell	Maris Piper	S.E.D.(1 d.f.)
Total yield (t/ha)	7.12	17.15	0.886
Ware yield (t/ha)	2.93	13.58	1.011
Percentage ware	30.5	74.7	3.78

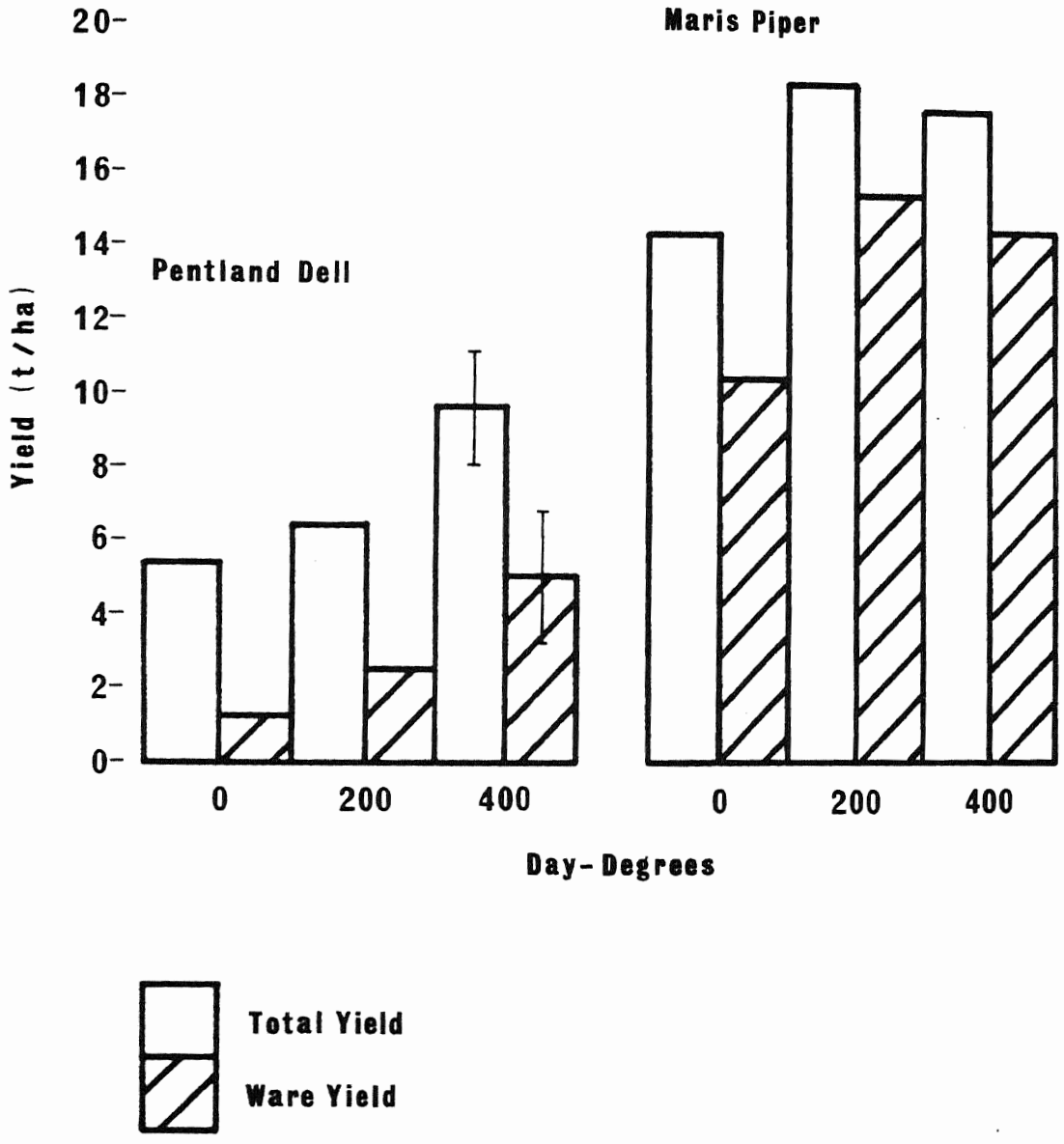
TABLE 9 Field Trial 1: Physiological age and yield

	Day-degrees			
	0	200	400	S.E.D.(2 d.f.)
Total yield (t/ha)	9.87	12.69	13.86	1.085
Ware yield (t/ha)	5.85	9.02	9.89	1.238
Percentage ware	40.5	52.5	64.7	4.63

TABLE 10 Field Trial 1: Physiological age, cultivar and yield

Cultivar	Day-degrees	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Pentland Dell	0	5.28	1.19	15.7
	200	6.32	2.54	28.2
	400	9.77	5.07	47.6
Maris Piper	0	14.45	10.51	65.4
	200	19.06	15.50	76.9
	400	17.95	14.71	81.8
S.E.D.(2 d.f.)		1.535	1.751	6.55

Fig.12 Field Trial 1: Cultivar, physiological age and yield



(Bars represent 95% confidence limits for total and ware yield)

TABLE 11 Field Trial 1: Ware yield of Pentland Dell as a percentage of Maris Piper

	Day-degrees		
	0	200	400
Maris Piper	100	100	100
Pentland Dell	11	16	34

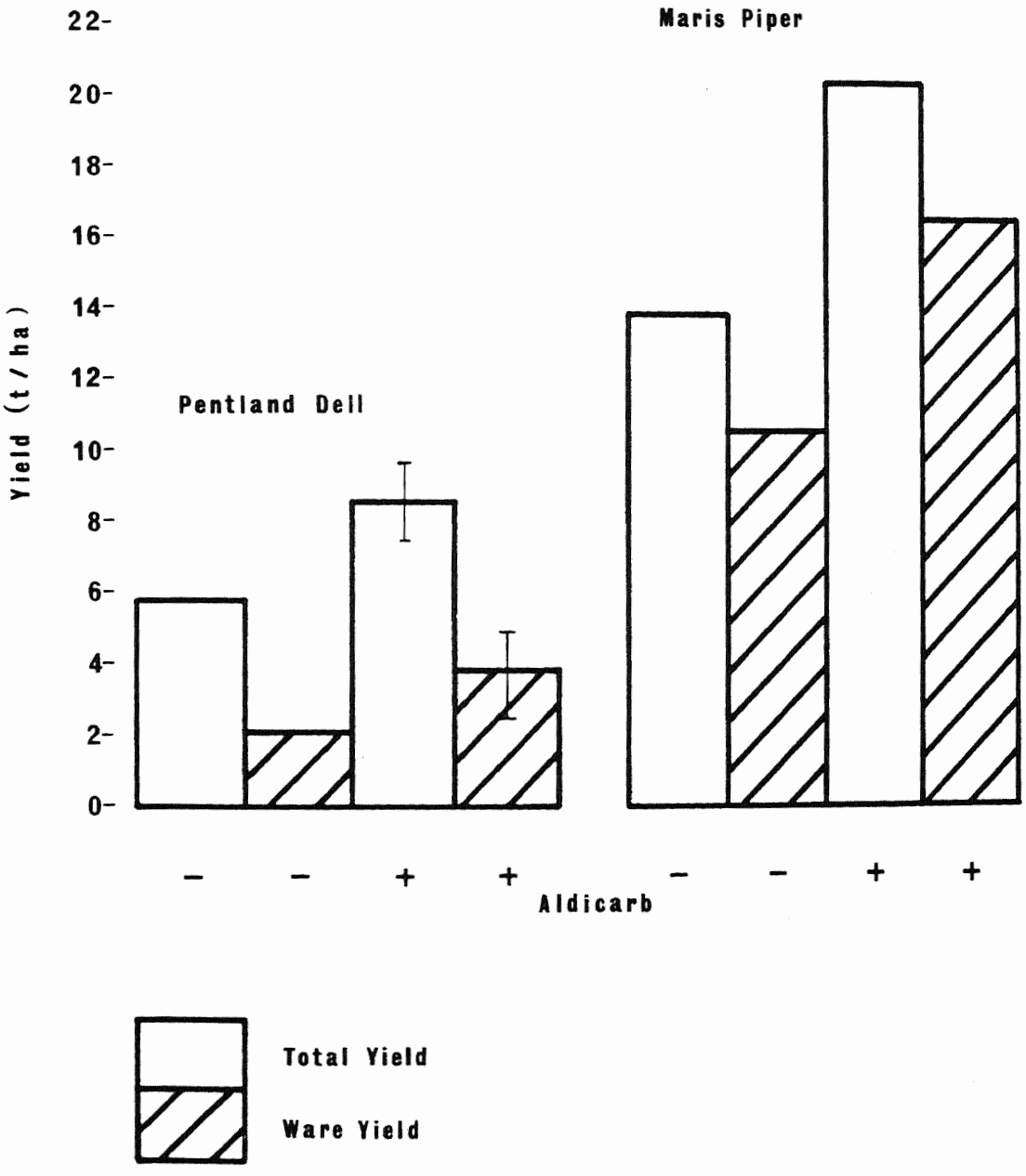
TABLE 12 Field Trial 1: Aldicarb and yield

	Aldicarb		
	-	+	S.E.D.(1 d.f.)
Total yield (t/ha)	9.85	14.43	0.778
Ware yield (t/ha)	6.26	10.25	0.689
Percentage ware	46.8	58.4	3.82

TABLE 13 Field Trial 1: Aldicarb, cultivar and yield

	Aldicarb	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
M. Piper	-	13.91	10.51	68.6
	+	20.40	16.64	80.7
P. Dell	-	5.79	2.01	24.9
	+	8.46	3.86	36.0
S.E.D.(1d.f.)		1.179	1.223	10.74

Fig.13 Field Trial 1: Cultivar, aldicarb and yield



(Bars represent 95% confidence limits for total and ware yields)

Nematode Population Densities and Multiplication Rates

Mean Pi, Pf and Pf/Pi data for each treatment are summarised in Table 14 . Overall mean Pi and Pf were 341 and 597 eggs/g soil, while the mean Pf/Pi ratio was 1.98.

There were no significant differences in Pi, Pf and Pf/Pi between cultivars, physiological ages and aldicarb treatment (Appendix 1).

It is important that mean Pi's are similar so that comparisons between physiological age and other treatments can be made at the same level of nematode stress. Nematode multiplication rates are density dependent (Seinhorst, 1966; Ferris, 1985; LaMondia and Brodie, 1986) and as Pi's were not significantly different we would not expect Pf's or Pf/Pi's to be significantly different between treatments as was the case in this field trial (Table 14).

Canopy Development

Mean percentage ground covers for each treatment from June to September are summarised in Table 15 . The data for aldicarb treated plots are shown graphically in Fig. 14. Both Maris Piper and Pentland Dell plants grown from physiologically old seed had larger canopies early in the growing season than plants grown from physiologically young seed. By late July there were no obvious effects of physiological age on canopy size with Maris Piper but in Pentland Dell plots physiologically old seed maintained larger canopies throughout the growth period. Maximum percentage ground covers were 42 and 62% for Pentland Dell and Maris Piper respectively; no plots reached full ground cover. The haulms of all treatments were stunted with pale green leaves, which are the classic symptoms of nutrient deficiency induced by nematode damage to the roots (Trudgill, 1980), (Plate 5).

Percentage ground cover duration (PGCD) was calculated by expressing percentage ground cover data from emergence to

TABLE 14 Field Trial 1: Mean Pi, Pf and Pf/Pi for all treatments

Cultivar	Day-degrees	Aldicarb	Pi	Pf	Pf/Pi
Pentland Dell	0	-	270	589	3.07
		+	298	447	1.74
	200	-	379	463	1.42
		+	393	599	1.55
	400	-	325	736	2.56
		+	331	624	1.87
Maris Piper	0	-	386	699	1.89
		+	291	552	1.91
	200	-	318	718	2.23
		+	496	514	1.10
	400	-	337	670	2.24
		+	270	556	2.14
S.E.D.(2 d.f.)			88.0	137.2	0.821

TABLE 15 Field Trial 1: Mean percentage ground covers for all treatments

		JUNE		JULY				AUGUST			SEPT
		25	30	7	14	21	28	4	11	25	8
		Days after planting									
Treatment		22	27	34	41	48	55	62	69	83	97
P. Dell	0 -A	0	2	10	19	13	16	14	15	12	12
	+A	2	7	13	16	13	28	12	13	9	4
	200 -A	1	6	10	9	9	20	8	7	3	3
	+A	4	7	20	26	25	42	21	25	16	15
	400 -A	5	10	16	18	11	18	13	11	15	11
	+A	5	15	23	30	25	37	27	28	30	22
M. Piper	0 -A	2	4	16	23	14	36	23	22	21	18
	+A	3	7	21	37	51	67	59	60	55	39
	200 -A	3	11	20	33	23	40	26	31	31	23
	+A	9	23	28	38	31	58	59	62	55	57
	400 -A	3	11	20	30	23	32	24	26	36	27
	+A	15	19	34	39	38	44	44	41	50	40

0,200,400 = day-degrees

-A = no aldicarb +A = aldicarb treated

Fig.14 Field Trial 1: Cultivar, aldicarb and percentage ground cover

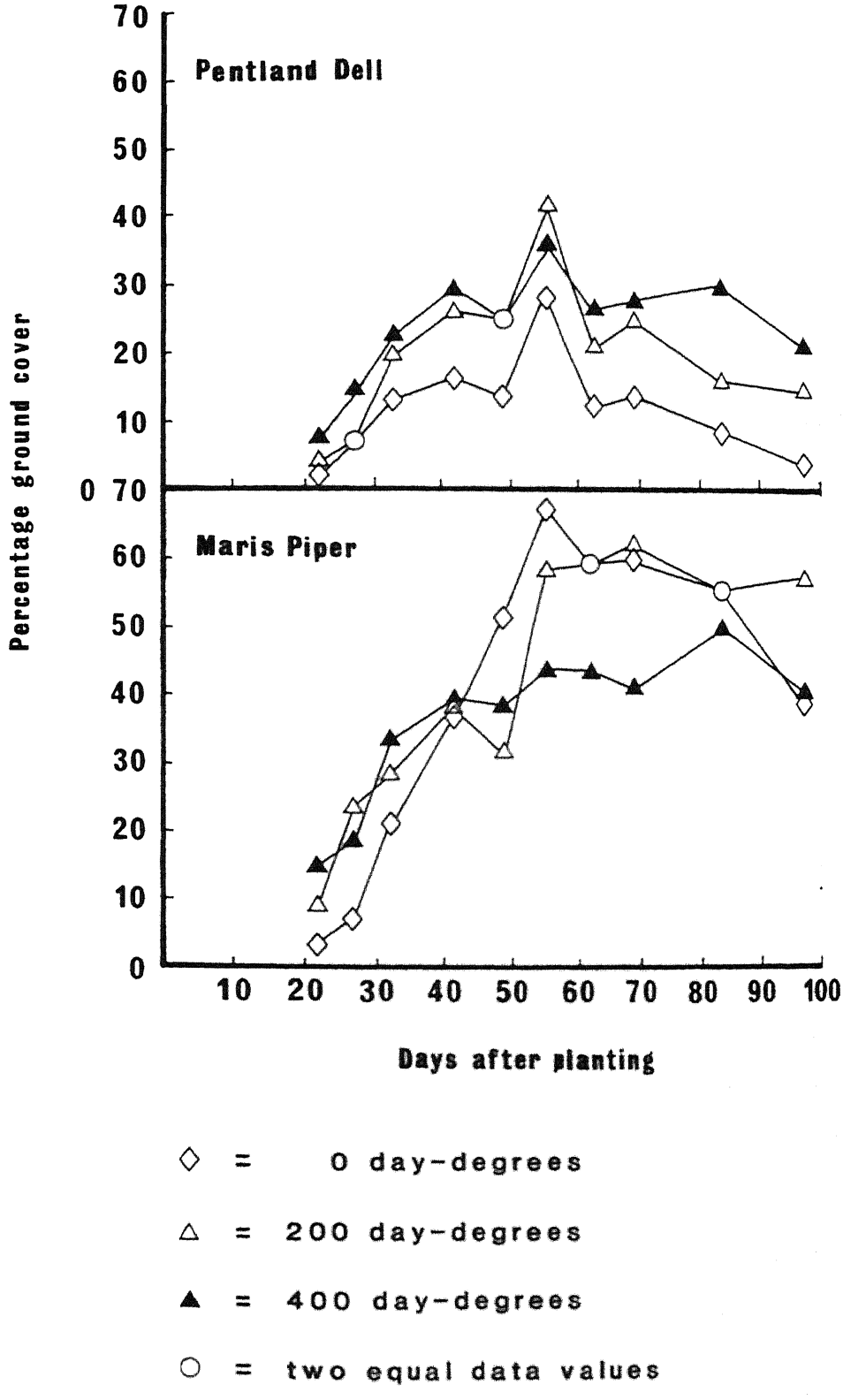


Plate 5 Field Trials 1 and 2

Field Trial 1 (July 1987)

Field Trial 2 (July 1987)

senescence as a percentage of maximum possible ground cover during this period i.e. 100% ground cover maintained from the date of crop emergence to the date of complete senescence/haulm desiccation.

PGCD for all plots was only 18.9%, so much incident radiation was falling onto bare ground. Maris Piper had a significantly higher PGCD than Pentland Dell ($P < 0.01$, Table 16) enabling it to intercept more solar radiation and produce larger yields than Pentland Dell (Table 8).

Plots grown from 400 day-degree seed tubers had higher PGCD's than those from 0 day-degree seed tubers (Table 17) contributing to larger yields (Table 9). However, for Pentland Dell, 400 day-degree plots had higher PGCD's than 0 day-degree plots; there was little difference between 0, 200 and 400 day-degrees for Maris Piper (Table 18 and Fig. 15).

Aldicarb treatment significantly increased PGCD overall ($P < 0.01$, Table 19) and when Maris Piper and Pentland Dell are considered individually ($P < 0.01$, Table 20) contributing to larger yields in aldicarb treated plots (Table 12 and Fig. 16).

Plant Dry Weights

Mean plant dry weights at the eight growth analysis sampling dates are summarised in Table 21. Dry weights from all treatments increase to a peak at 83 days after planting (late August) and then decline (Table 22). At all sampling dates Maris Piper plants were significantly heavier than those of Pentland Dell ($P < 0.01$, Table 23), with both cultivars reaching their maximum dry weight at the end of August. This corresponds with canopy development in both cultivars which was maximum in early to mid August, after which the canopies senesced (Fig. 14).

From 27 to 69 days after planting (late June to mid August) plants grown from 400 and 200 day-degree seed tubers were significantly heavier than those from 0 day-degree seed tubers

TABLE 16 Field Trial 1: Cultivar and PGCD

	Pentland Dell	Maris Piper	S.E.D.(1 d.f.)
PGCD	11.9	25.9	1.30

TABLE 17 Field Trial 1: Physiological age and PGCD

	Day-degrees			
	0	200	400	S.E.D.(2 d.f.)
PGCD	16.8	19.4	20.5	1.59

TABLE 18 Field Trial 1: Cultivar, physiological age and PGCD

	Day-degrees			
	0	200	400	S.E.D.(2 d.f.)
Pentland Dell	9.3	11.0	15.5	2.25
Maris Piper	24.4	27.8	25.5	2.25

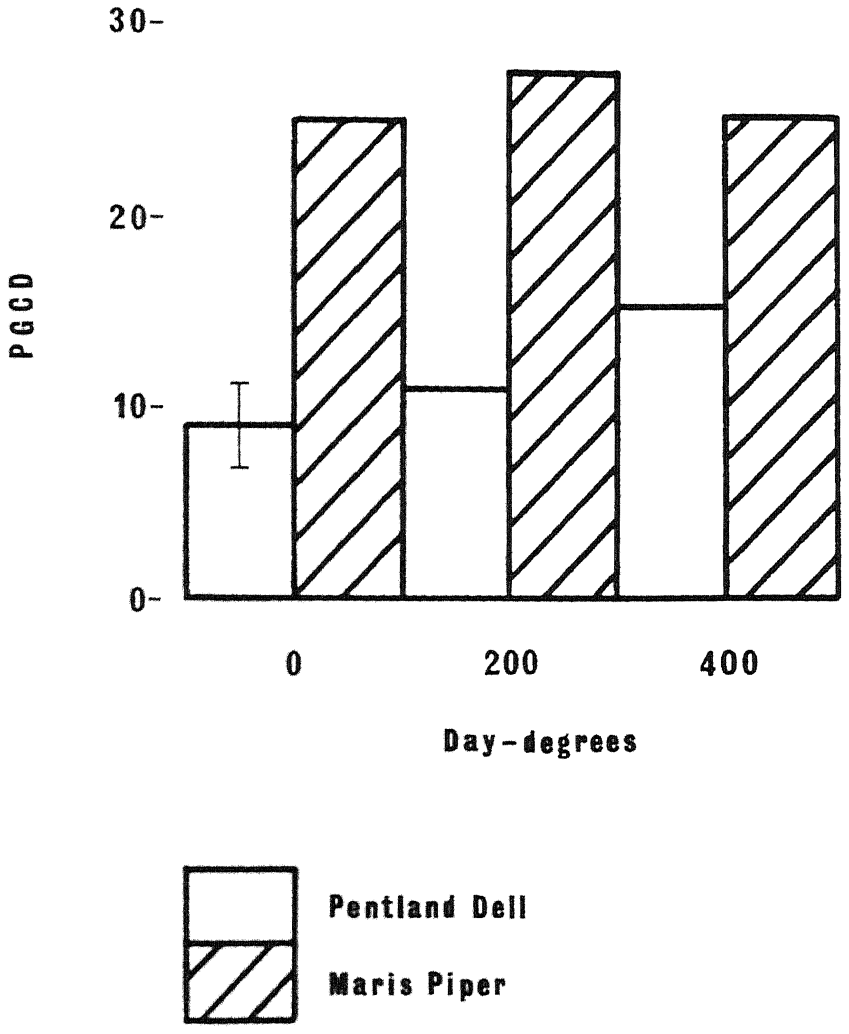
TABLE 19 Field Trial 1: Aldicarb and PGCD

	Aldicarb		
	-	+	S.E.D.(1 d.f.)
PGCD	13.4	24.4	1.34

TABLE 20 Field Trial 1: Cultivar, aldicarb and PGCD cover

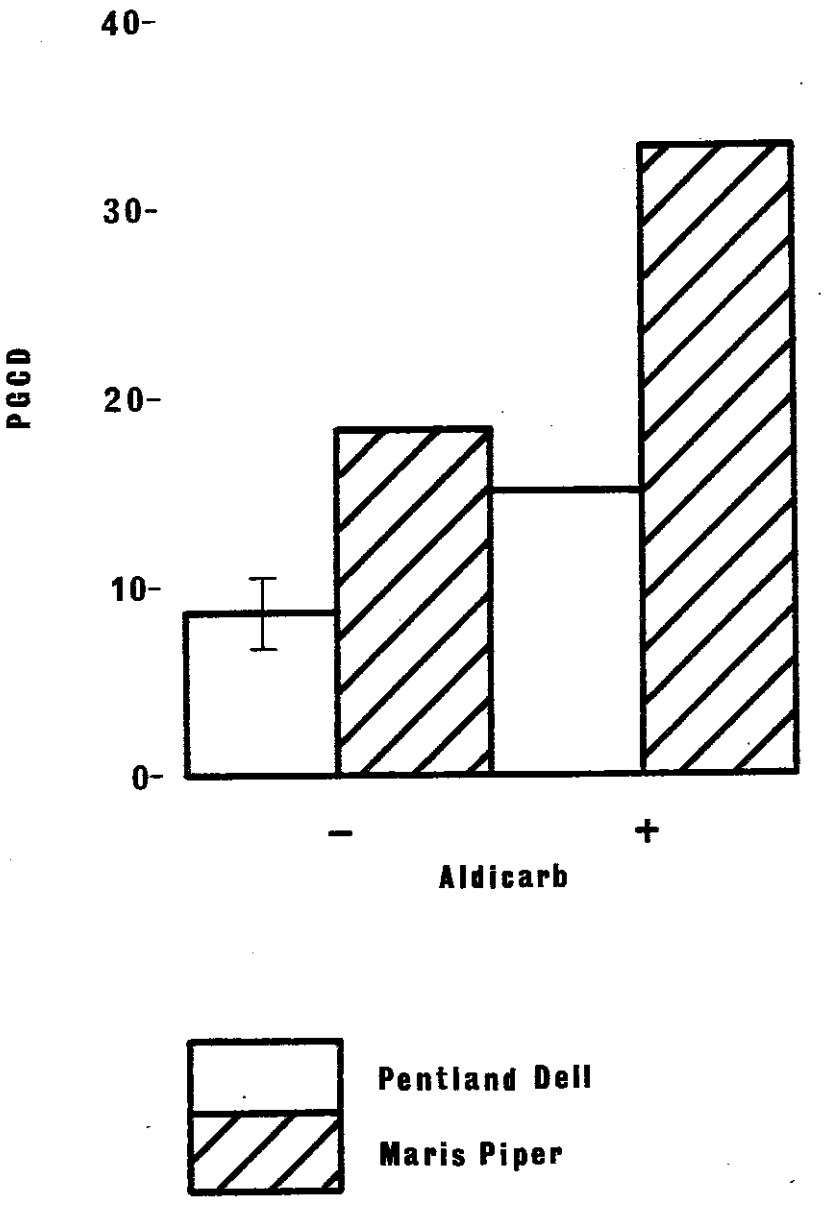
	Aldicarb		
	-	+	S.E.D.(1 d.f.)
Pentland Dell	8.6	15.2	1.87
Maris Piper	18.3	33.5	

Fig.15 Field Trial 1: Cultivar, physiological age and PGCD



(Bar represents 95% confidence limits for all treatments)

Fig.16 Field Trial 1: Cultivar, aldicarb and PGCD



(Bar represents 95% confidence limits for all treatments)

TABLE 21 Field Trial 1: Mean plant dry weights (g) for all treatments

		JUNE		JULY		AUGUST			SEPT
		30	7	14	28	4	11	25	8
		Days after planting							
Treatment		27	34	41	55	62	69	83	97
P. Dell	0 -A	1.2	3.2	4.8	7.3	14.4	17.3	35.6	34.4
	+A	1.1	3.6	7.4	11.2	13.7	18.2	44.0	19.5
	200 -A	1.3	3.8	4.2	10.2	14.0	11.8	34.3	22.7
	+A	1.7	4.5	9.0	17.2	25.2	33.0	102.7	44.1
	400 -A	2.2	4.4	7.7	19.4	27.8	37.3	55.3	49.8
	+A	2.6	4.8	10.3	20.5	32.4	42.7	99.5	55.0
M. Piper	0 -A	1.8	3.2	7.1	23.4	35.3	29.0	84.2	61.5
	+A	2.1	5.1	11.2	40.5	43.2	53.8	152.2	127.5
	200 -A	3.0	6.6	15.8	38.6	50.9	73.4	167.7	127.6
	+A	4.4	8.4	21.0	39.7	78.1	75.2	249.4	164.3
	400 -A	2.9	6.8	16.1	41.8	55.9	84.8	238.5	117.3
	+A	3.4	8.8	16.8	40.4	71.0	66.3	197.0	125.4
S.E.D.(2 d.f.)		0.55	1.64	2.33	6.48	8.18	9.35	61.83	26.01

0,200,400 = day-degrees

-A = no aldicarb +A = aldicarb treated

TABLE 22 Field Trial 1: Mean plant dry weights of all treatments

Days after planting	Mean plant dry weight (g)
27	2.31
34	5.27
41	10.95
55	25.85
62	38.48
69	45.22
83	121.69
97	79.08

TABLE 23 Field Trial 1: Cultivar and plant dry weight (g)

Days after planting	Pentland Dell	Maris Piper	S.E.D.(1 d.f.)
27	1.70	2.91	0.254
34	4.03	6.52	0.551
41	7.24	14.66	0.780
55	14.29	37.41	2.713
62	21.24	55.73	3.931
69	26.71	63.73	4.120
83	61.89	181.50	30.231
97	37.57	120.59	12.537

($P < 0.10$); there were no significant differences in dry weights between 200 and 400 day-degrees. However, after mid August these differences were no longer significant (Table 24). The effect of seed tuber physiological age on the plant dry weight of Maris Piper and Pentland Dell individually is shown in Table 25 and in Fig. 17. For Maris Piper, differences between 200 and 400 day-degrees were not significant but both had significantly heavier dry weights than plants grown from 0 day-degree seed tubers. At all harvesting dates, Pentland Dell plants grown from 400 day-degree seed tubers were heaviest and 0 day-degree lightest, with 200 day-degree intermediate. However, these differences were not significant at any sampling date between 0 and 200 day-degrees or at 34, 83 and 97 days after planting between 0 and 400 day-degree seed tubers.

Treatment with aldicarb increased plant dry weight at all sampling dates (Table 26) but these differences were not significant at 34, 55 and 69 days after harvest.

Nematodes in the Roots

The number of nematodes (all developmental stages) in the roots of plants sampled at 27, 55 and 97 days after planting, expressed as nematodes per g of root and nematodes per root system, are summarised for each treatment in Table 27.

At 27 days after planting and when all treatments are considered together there was an average of 17,478 nematodes per root system, representing a nematode density of 2994 nematodes per g of root (Table 28). Such large nematode burdens injure the roots and stunt their growth, resulting in a diminished supply of water and nutrients to the stems and leaves. This is a major contributive factor to the small canopies and stunted appearance of plants in this field trial. Numbers of nematodes in the roots decline from the first to the last sampling date (Table 28) as the JJ2 that have invaded the roots develop into mature females

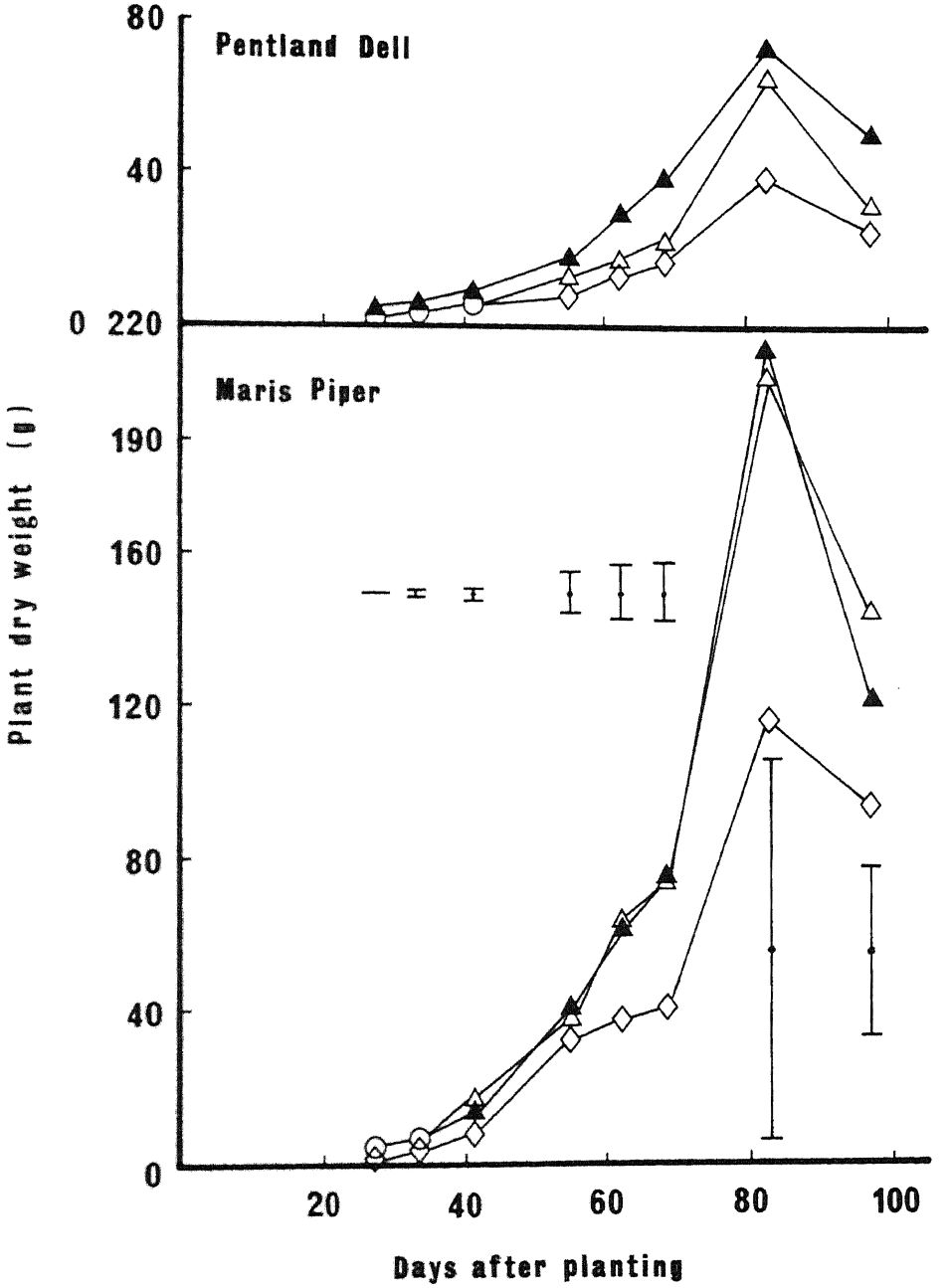
TABLE 24 Field Trial 1: Physiological age and plant dry weight(g)

Days after planting	Day-degrees 0	200	400	S.E.D.(2 df)
27	1.56	2.61	2.76	0.311
34	3.77	5.84	6.22	0.675
41	7.62	12.50	12.72	0.955
55	20.60	26.43	30.52	3.323
62	26.66	42.04	46.76	4.814
69	29.57	48.34	57.76	5.045
83	78.97	138.54	147.57	37.026
97	60.72	89.67	86.86	15.354

TABLE 25 Field Trial 1: Cultivar, physiological age and plant dry weight (g)

	Physiological age (day-degrees)						
	0		200		400		
Days after planting	P Dell	M Piper	P Dell	M Piper	P Dell	M Piper	S.E.D. (2 df)
27	1.18	1.93	1.51	3.70	2.40	3.12	0.439
34	3.37	4.17	4.14	7.54	4.60	7.83	0.955
41	6.11	9.14	6.59	18.42	9.02	16.43	1.351
55	9.26	31.94	13.70	39.17	19.91	41.12	4.699
62	14.04	39.28	19.59	64.48	30.08	63.43	6.808
69	17.77	41.37	22.39	74.29	39.98	75.53	7.135
83	39.79	118.16	68.50	208.59	77.39	217.74	52.362
97	26.97	94.48	33.39	145.94	52.36	121.36	21.714

Fig.17 Field Trial 1: Cultivar and plant dry weight during the growing season



◇ = 0 day-degrees

△ = 200 day-degrees

▲ = 400 day-degrees

○ = two similar data values

(bars represent 95% confidence limits for both cultivars)

TABLE 26 Field Trial 1: Aldicarb and plant dry weight (g)

Days after planting	Aldicarb -	Aldicarb +	S.E.D.(1 d.f.)
27	2.06	2.55	0.195
34	4.67	5.88	0.771
41	9.29	12.61	1.094
55	23.44	28.26	2.573
62	33.05	43.92	2.618
69	42.24	48.21	3.488
83	102.59	140.80	18.981
97	68.87	89.30	8.261

TABLE 27 Field Trial 1: Mean nematode root populations for all treatments

Treatment			Days after planting					
			27	55	97	27	55	97
			Nematodes/g of root			Nematodes/root system		
P. Dell	0	-A	3010	870	284	12000	4611	403
		+A	2168	741	267	9300	4939	262
	200	-A	5153	930	267	27000	4921	490
		+A	2348	771	134	9400	5301	529
	400	-A	3584	746	168	23000	7630	599
		+A	3003	870	237	19000	6989	513
M. Piper	0	-A	2775	665	156	15000	7247	591
		+A	2527	320	68	11000	3293	525
	200	-A	3170	340	127	24000	3293	760
		+A	2188	437	78	18000	2826	724
	400	-A	3264	336	99	23000	3904	739
		+A	2734	264	116	19000	3430	946
S.E.D.(2 d.f.)			556.4	164.9	90.9	5219.4	1432.2	217.9

0,200,400 = day-degrees

-A = no aldicarb +A = aldicarb treated

TABLE 28 Field Trial 1: Mean nematode root populations of all treatments

	Days after planting	No. of nematodes
Nematodes/g of root	27	2994
	55	603
	97	167
Nematodes/ root system	27	17478
	55	4865
	97	590

and males and ultimately enter the rhizosphere.

The roots of Pentland Dell contained significantly more nematodes per g than Maris Piper at each sampling date ($P < 0.05$, Table 29). At 27 days after planting there was no significant difference in the total number of nematodes per root system between cultivars. By 55 days after planting, Pentland Dell contained significantly more nematodes per root system than Maris Piper ($P < 0.05$); at 97 days this situation was reversed ($P < 0.05$, Table 29).

At 27 days after planting the roots of plants grown from 200 and 400 day- degree seed tubers contained significantly larger numbers of nematodes per g and nematodes per root system than the roots of 0 day-degree plants ($P<0.05$, Table 30). There were no significant differences between 200 and 400 day-degree plants. After 27 days, numbers of nematodes did not differ significantly between physiological age treatments (Table 30). Within cultivars, the only significant effect of physiological age on the number of nematodes per g of root was for Pentland Dell, which had greater numbers in 400 than 0 day-degree roots at 27 days after planting ($P<0.10$, Table 31). Roots of Pentland Dell plants grown from 400 day-degree seed tubers had significantly more nematodes per root system than those grown from 0 day-degree seed tubers at 27 and 55 days after planting; at 55 days, 400 day-degree roots contained more nematodes per root system than 200 day-degree roots. The only significant difference for Maris Piper was at 55 days after planting when 200 day-degree roots contained less nematodes per root system than 0 day-degree roots ($P<0.10$, Table 31).

Treatment of plots with aldicarb reduced the number of nematodes per g of root and the number of nematodes per root system at 27, 55 and 97 days after planting. However, this difference was only significant at 27 days ($P < 0.05$, Table 32).

TABLE 29 Field Trial 1: Cultivar and nematode root populations

	Days after planting	P. Dell	M. Piper	S.E.D.(1 df)
Nematodes/g of root	27	3211	2776	173.8
	55	822	394	60.5
	97	226	107	43.5
Nematodes/ root system	27	16000	18000	2105.4
	55	5732	3999	566.0
	97	466	714	110.4

TABLE 30 Field Trial 1: Physiological age and nematode root populations

	Days after planting	Day-degrees			S.E.D(2df)
		0	200	400	
Nematodes/g of root	27	2620	3215	3146	212.9
	55	649	620	554	74.1
	97	194	151	155	53.2
Nematodes/ root system	27	12000	20000	21000	2578.6
	55	5022	4085	5488	693.2
	97	445	626	699	135.2

TABLE 31 Field Trial 1: Cultivar, physiological age and nematode root populations

		Day-degrees						
		0		200		400		
	Days	P Dell	M Piper	P Dell	M Piper	P Dell	M Piper	SED(2df)
Nems/ g root	27	2589	2651	3750	2679	3293	2999	301.1
	55	806	493	851	389	808	300	104.8
	97	275	112	200	102	202	107	75.3
Nems/ root system	27	11000	13000	18000	21000	21000	21000	3646.7
	55	4775	5270	5111	3060	7309	3667	980.3
	97	333	558	509	742	556	843	191.2

TABLE 32 Field Trial 1: Aldicarb and nematode root populations

	Days after planting	Aldicarb		S.E.D.(1 df)
		-	+	
Nematodes/g root	27	3493	2494	270.2
	55	648	567	73.5
	97	183	150	29.4
Nematodes/ root system	27	21000	14000	2155.9
	55	5268	4463	602.8
	97	597	583	60.4

Aldicarb inhibits the movement and feeding of nematodes by its deleterious effect on acetylcholinesterase at cholinergic synapses in the nervous system (Evans, 1973). Aldicarb has been shown to reduce the hatching of G. rostochiensis (Osbourne, 1973) and JJ2 mobility once hatched (Nelmes, 1970). These effects combine to reduce the invasion of potato roots by PCN (Whitehead, 1973) and explain the reduced numbers of nematodes in the roots of aldicarb treated plants.

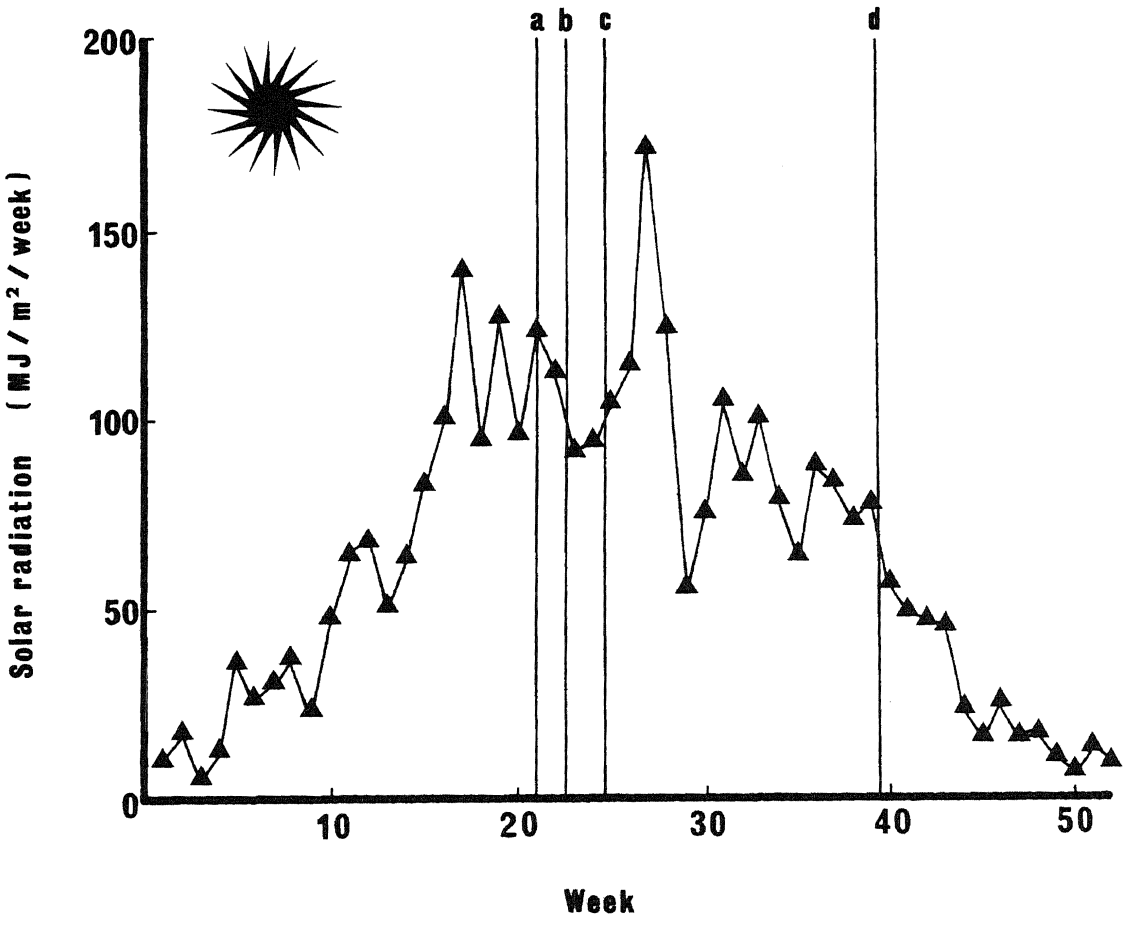
3.2.4 Conclusions

Tuber yields were low (mean ware yield 8.25 t/ha) as a result of the damage to the plants' roots inflicted by a moderately high nematode population density (mean Pi=341 eggs per g soil) inhibiting the uptake of water and nutrients, resulting in stunted canopies which could only intercept a small amount of incident radiation (maximum PGCD = 18.9%). The maximum amount of incident radiation that could have been intercepted (and hence potential tuber yield) was decreased by the late planting date (2 and 3 of June) caused by the delayed delivery of seed tubers from Scotland. Incident solar radiation was near its peak when this field trial was planted (Fig. 18 and Appendix 2) and earlier planting would have had a large effect on the maximum amount of radiation that could have been intercepted by the crop canopy.

The moderately high Pi was uniform between plots and nematode multiplication rates were low (mean Pf/Pi = 1.98) due to the density dependent effect of a high initial population density. Cultivar, physiological age and aldicarb treatment had no significant overall effect on Pf or Pf/Pi.

Maris Piper plants produced greater tuber yields than Pentland Dell and were therefore more tolerant of nematode attack. Nematode population densities in the roots were high and declined during the growing season as nematodes matured and left the roots. The roots of Pentland Dell plants contained higher

Fig.18 Incident solar radiation 1987 (Broom's Barn)



a = Field Trial 2, planting date 1

b = Field Trial 1 planted

c = Field Trial 2, planting date 2

d = Harvest

nematode population densities through the growing season than Maris Piper but there were no clear trends in the total numbers of nematodes in the root systems between cultivars. The canopies of Maris Piper were larger than Pentland Dell and reached higher peak percentage ground cover. Plant dry weights increased to a peak in late August after which they declined due to the onset of senescence. Maris Piper plants were heavier than those of Pentland Dell. From planting to harvest, Maris Piper had a higher PGCD than Pentland Dell, enabling it to intercept more solar radiation and produce higher tuber yields than Pentland Dell.

Plants grown from seed tubers conditioned to 400 day-degrees produced higher tuber yields than those from 0 day-degree seed tubers and this effect was greater for Pentland Dell than for Maris Piper. Early in the growing season (27 days after planting) the roots of 200 and 400 day-degree plants contained a higher nematode density and total number of nematodes than 0 day-degree plants. After this time there were no differences in numbers of nematodes. The canopies of both cultivars grown from physiologically aged seed were larger at the beginning of the season than those grown from unaged seed. This advantage to aged seed was maintained throughout the season by Pentland Dell. Plant dry weight increased with increasing physiological age until mid August, after which the effect was no longer significant. Overall the PGCD of 400 day-degree plots was greater than in 0 day-degree plots, enabling plants grown from physiologically old seed to intercept more solar radiation and produce higher tuber yields than those grown from 'young' seed.

Aldicarb treatment increased the yield of both cultivars. The roots of aldicarb treated plants had lower nematode population densities and total numbers of nematodes. Reduced nematode stress on the plants enabled them to support larger canopies, resulting in higher dry weights and increased PGCD

compared to untreated plots.

3.3 FIELD TRIAL 2

The effect of seed tuber physiological age, planting date and nematicide treatment on the growth and yield of the first early cultivar Maris Bard, second early Estima and maincrop cultivars Maris Piper and Pentland Dell.

3.3.1 Experimental Design

This trial was sited within grids 18-20 of the preliminary survey of Field No 1 with a mean estimated Pi of 400 eggs/g soil (Fig 9).

Seed tubers of Maris Bard, Estima, Maris Piper and Pentland Dell were conditioned to two physiological ages (0 and 400 day-degrees above 4°C) and planted at two different dates in plots split for nematicide treatment.

The trial consisted of three blocks each containing 32 split plots (Fig 19) where a split plot contained a harvest row between two guard rows. There were 10 plants in a row guarded at each end by two Desiree plants (Fig 20).

3.3.2 Methods

Physiological ageing of seed tubers, nematicide application, weed and disease control, measurement of canopy development, soil sampling and harvesting were carried out in the same way as for Field Trial 1.

Planting

On 21 May and 11 June seed tubers were planted by hand to a depth of approximately 5cm into fully formed ridges. Seed tubers that were not required for the first planting date were kept at 3C until 11 June.

Growth Analysis

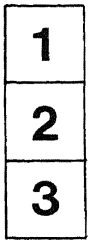
Two plants were carefully removed from each plot from the first and second planting dates on 1 July and 30 July

FIG. 19 Field Trial 2: Experimental design

One block (all three blocks identical):-

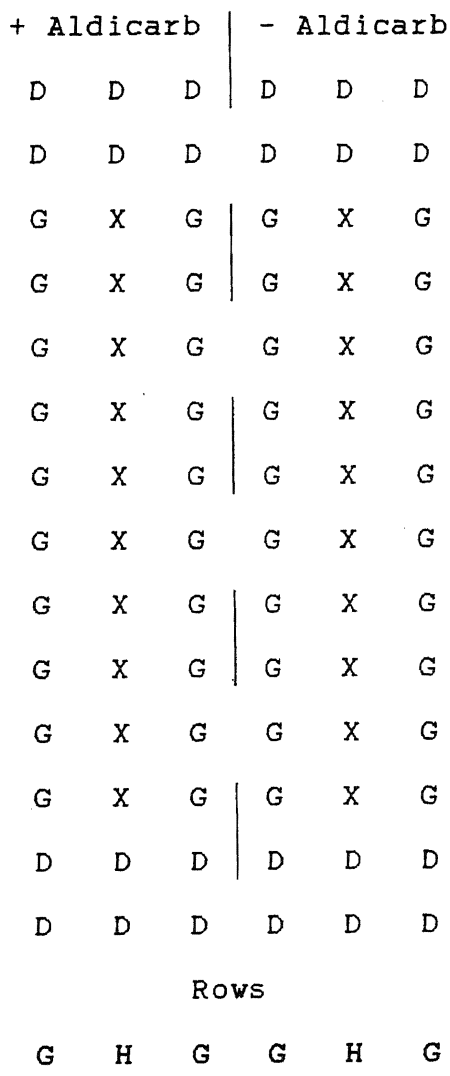
Maris Piper 0 d.d. 1 -N +N	Estima 0 d.d. 2 -N +N	Estima 0 d.d. 1 -N +N	Pentland Dell 0 d.d. 2 -N +N
Estima 0 d.d. 2 -N +N	Maris Bard 400 d.d. 2 +N -N	Pentland Dell 400 d.d. 1 +N -N	Maris Bard 0 d.d. 1 -N +N
Pentland Dell 0 d.d. 1 -N +N	Pentland Dell 400 d.d. 2 +N -N	Estima 400 d.d. 1 +N -N	Maris Piper 400 d.d. 2 +N -N
Maris Piper 0 d.d. 2 -N +N	Maris Bard 400 d.d. 1 +N -N	Maris Piper 400 d.d. 1 +N -N	Maris Bard 0 d.d. 2 -N +N

+N = 4.4 kg/ha aldicarb
-N = no aldicarb
d.d. = day-degrees
1,2 = planting dates



Block layout

FIG. 20 Field Trial 2 : Split plot design



H = Harvest row

G = Guard row

X = Harvest plant

D = Desiree guard plant

G = Guard plant (same seed as harvest plant)

respectively (6 weeks after planting). The plants were dissected and the following data recorded:-

1) The fresh weight of haulm

2) The fresh weight of subterranean stems and stolons

3) The fresh weight of tubers of diameter at least 10mm

4) The fresh weight of the root system

A 2g sub sample of the root system was preserved in FAA.

3.3.3 Results and Discussion

Yield

Mean total, ware and percentage ware yield for each treatment are summarised in Table 33. Tuber yields from this trial were low with a mean ware yield of 10.96t/ha accounting for 64.3% of the total yield of 14.68t/ha.

Maris Piper produced the greatest total and ware yield while Pentland Dell had the least ($P< 0.01$); Maris Bard and Estima were intermediate in yield but were not significantly different from each other (Table 34).

Physiological age had no significant effect on overall yields (Appendix 3). When individual cultivars were considered the only significant effect was in Maris Bard plots, where plants grown from 0 day-degree seed tubers had a larger total yield than those from 400 day-degree seed tubers ($P< 0.10$, Table 35).

Planting date and aldicarb treatment had no significant effect on overall yields or the yields of individual cultivars (Appendix 3).

Nematode Population Densities and Multiplication Rates

Mean Pi, Pf and Pf/Pi data for each treatment are summarised in Table 36. Overall mean Pi and Pf were 347 and 645 eggs/g soil, while the mean Pf/Pi ratio was 2.33.

There were significant differences in Pi's between cultivars. The Pi in Pentland Dell plots was significantly higher than in those of Estima, Maris Piper and Maris Bard ($P< 0.10$).

TABLE 33 Field Trial 2: Mean yields for all treatments

Day-degrees		0				400			
Planting date		1		2		1		2	
Aldicarb		0	4.4	0	4.4	0	4.4	0	4.4
Total yield (t/ha)	E	10.98	16.79	20.60	17.71	11.80	16.14	13.00	13.60
	MP	23.04	26.73	17.76	19.17	24.57	23.19	25.13	24.55
	MB	16.83	13.89	17.38	13.28	7.74	8.00	12.91	10.97
	PD	8.03	8.97	9.09	6.70	7.49	10.68	6.81	6.29
Ware yield (t/ha)	E	7.47	13.09	15.68	13.22	8.86	13.35	10.27	9.83
	MP	20.34	23.78	13.53	14.44	22.18	19.83	22.74	21.21
	MB	13.75	11.28	9.92	7.93	4.92	5.92	11.03	8.46
	PD	3.04	4.21	2.49	2.45	4.79	6.51	1.91	2.25
Percent ware	E	52.8	71.8	76.1	74.1	62.5	78.8	77.6	65.0
	MP	88.2	89.0	75.7	74.8	88.0	83.9	90.0	84.1
	MB	69.8	74.9	53.0	57.3	54.4	73.6	84.7	76.8
	PD	37.9	47.5	19.0	19.3	47.0	49.2	26.6	35.1

S.E.D.(3 d.f.) Total yield = 3.588
Ware yield = 3.698
Percentage ware = 13.11

E = Estima; MP = Maris Piper; MB = Maris Bard; PD = Pentland Dell

TABLE 34 Field Trial 2: Cultivar mean yields

	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Estima	15.08	11.47	69.8
Maris Piper	23.02	19.76	84.2
Maris Bard	12.63	9.15	68.1
Pentland Dell	8.01	3.45	35.2
S.E.D.(3 d.f.)	1.587	1.660	5.91

TABLE 35 Field Trial 2: Physiological age, cultivarand yield

Cultivar	Day-degrees	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Estima	0	16.52	12.37	68.7
	400	13.63	10.58	71.0
Maris Piper	0	21.67	18.02	81.9
	400	24.36	21.49	86.5
Maris Bard	0	15.34	10.72	63.8
	400	9.91	7.59	72.4
Pentland Dell	0	8.20	3.05	30.9
	400	7.82	3.86	39.5
S.E.D.(3 d.f.)		2.244	2.347	8.36

TABLE 36 Field Trial 2: Mean Pi, Pf and Pf/Pi for all treatments

Day-degrees		0				400				
Planting date		1		2		1		2		
Aldicarb		-	+	-	+	-	+	-	+	SED(3 df)
	Pi	460	316	165	218	378	549	216	340	99.7
Estima	Pf	683	848	714	904	891	910	905	639	152.6
	Pf/Pi	1.44	2.98	5.40	4.34	2.35	1.78	4.33	2.17	1.210
	Pi	186	239	198	224	410	443	392	385	99.7
M Piper	Pf	603	574	609	622	783	597	955	725	152.6
	Pf/Pi	3.90	5.28	3.74	3.00	2.27	1.34	2.34	1.91	1.210
	Pi	304	380	422	462	281	210	311	246	99.7
M Bard	Pf	473	579	511	634	416	423	521	565	152.6
	Pf/Pi	1.60	1.62	1.25	1.51	1.56	2.03	1.73	2.38	1.210
	Pi	203	289	544	496	472	568	464	322	99.7
P Dell	Pf	493	589	516	783	567	688	442	469	152.6
	Pf/Pi	2.72	2.05	0.94	1.62	1.23	1.22	1.01	1.57	1.210

The Pf in Estima plots was significantly higher than the other cultivars; Pf in Maris Piper plots was higher than in Maris Bard plots but neither were significantly greater than Pentland Dell plots. As a result of the density dependent nature of PCN multiplication, Estima had the highest Pf/Pi ratio which was significantly greater than those of Maris Bard and Pentland Dell (Table 37).

When physiological age is considered there were no overall differences in Pi, Pf and Pf/Pi between 0 and 400 day-degree plots (Appendix 4). However, within Maris Piper treatments 400 day-degree plots had higher Pi's than 0 day-degree plots (Table 38).

Aldicarb treatment had no overall effect on Pi, Pf or Pf/Pi or when individual cultivars were considered (Appendix 4). Planting date had no overall effect on Pi, Pf or Pf/Pi (Appendix 4) but the first planting date for Estima had a significantly higher Pi than the second planting date (Table 39).

Plant Emergence and Canopy Development

Percentage plant emergence data for each treatment 21 days after the first planting date are shown in Table 40. Emergence from 0 day-degree seed is negligible for all cultivars while for 400 day-degree seed there is 20-40% emergence in Maris Piper, Maris Bard and Pentland Dell plots and Estima has 60-70% emergence.

Mean percentage ground covers from late June to September are summarised in Table 41. The data for first planting date aldicarb treated plots are shown graphically in Fig 21. In all cultivars plants grown from physiologically old seed had larger canopies early in the growing season than plants grown from physiologically young seed. At 50-70 days after planting date 1 there is little difference in percentage ground cover between physiological ages within a cultivar. Plants grown from 400 day-

TABLE 37 Field Trial 2: Cultivar and Pi, Pf and Pf/Pi

	Estima	M. Piper	M. Bard	P. Dell	S.E.D.(3 d.f.)
Pi	330	310	327	420	43.6
Pf	812	683	515	569	59.1
Pf/Pi	3.10	2.97	1.71	1.54	0.488

TABLE 38 Field Trial 2: Cultivar, physiological age and Pi, Pf and Pf/Pi

	Day-degrees	Pi	Pf	Pf/Pi
Estima	0	290	787	3.54
	400	371	836	2.66
Maris Piper	0	212	602	3.98
	400	408	765	1.97
Maris Bard	0	392	549	1.49
	400	262	481	1.93
Pentland Dell	0	383	595	1.83
	400	457	542	1.26
S.E.D.(3 d.f.)		61.7	83.5	0.691

TABLE 39 Field Trial 2: Cultivar, planting date and Pi, Pf and Pf/Pi

	Planting date	Pi	Pf	Pf/Pi
Estima	1	426	833	2.14
	2	235	790	4.06
Maris Piper	1	320	639	3.20
	2	300	728	2.75
Maris Bard	1	294	473	1.70
	2	360	558	1.72
Pentland Dell	1	383	584	1.80
	2	457	553	1.29
S.E.D.(3 d.f.)		61.7	83.5	0.691

TABLE 40 Field Trial 2: Percentage plant emergence on 11 June (21 days after the first planting date)

	Day-degrees	Aldicarb	
		-	+
Estima	0	1	3
	400	68	64
Maris Piper	0	0	0
	400	29	39
Maris Bard	0	0	0
	400	20	22
Pentland Dell	0	0	0
	400	23	29

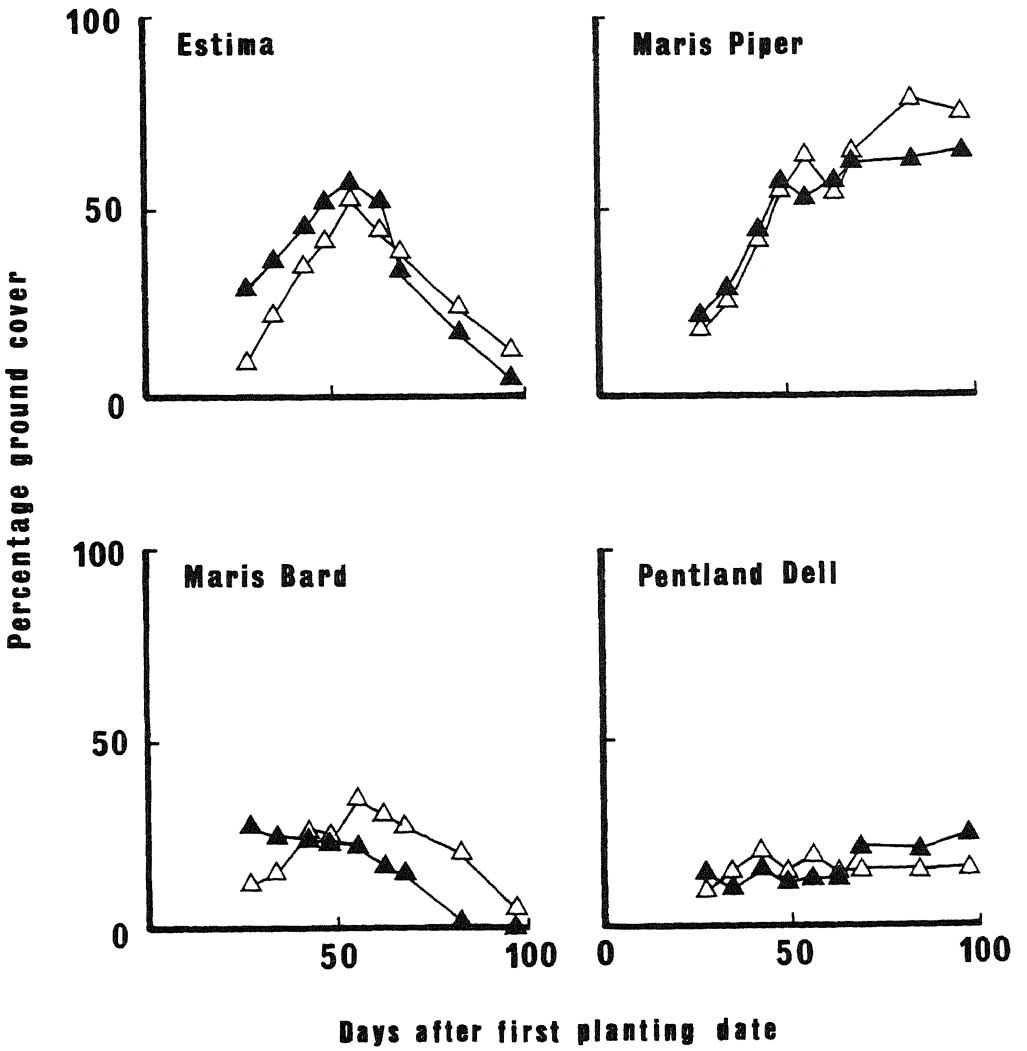
TABLE 41 Field Trial 2: Mean percentage ground covers for all treatments

			JUNE		JULY			AUGUST			SEPT
			30	7	14	21	28	4	11	25	8
			Days after planting								
Treatment			27	34	41	48	55	62	69	83	97
Estima	0	-A 1	6	13	22	27	30	30	25	24	19
		+A 1	10	22	35	41	53	44	39	24	12
		-A 2	1	6	23	31	39	37	40	57	48
		+A 2	0	7	19	25	40	37	38	44	29
	400	-A 1	14	24	37	32	40	26	21	14	4
		+A 1	29	37	46	52	58	52	35	18	6
		-A 2	5	15	30	29	37	23	27	21	12
		+A 2	5	14	32	30	40	28	33	29	16
M Piper	0	-A 1	14	18	37	38	54	49	55	73	67
		+A 1	18	26	40	54	63	53	65	79	76
		-A 2	1	8	20	18	27	28	29	43	58
		+A 2	1	6	14	23	25	25	28	42	58
	400	-A 1	10	33	35	34	41	35	41	56	58
		+A 1	21	29	42	55	52	56	63	61	65
		-A 2	4	12	28	27	43	38	37	53	58
		+A 2	5	23	31	30	40	37	45	53	61
M Bard	0	-A 1	11	17	32	32	52	35	32	23	5
		+A 1	12	16	26	23	34	30	28	20	7
		-A 2	0	2	15	14	31	24	25	28	17
		+A 2	0	1	10	14	25	20	23	24	19
	400	-A 1	24	21	26	18	23	12	10	0	0
		+A 1	28	25	25	25	22	17	16	1	0
		-A 2	6	13	25	31	33	27	29	22	10
		+A 2	5	10	21	27	32	22	22	18	3
P Dell	0	-A 1	13	13	18	19	17	15	15	18	20
		+A 1	10	16	20	16	19	16	16	16	16
		-A 2	0	3	18	16	23	16	14	18	14
		+A 2	0	5	10	8	15	10	13	12	11
	400	-A 1	4	9	12	9	13	9	13	14	14
		+A 1	24	20	27	21	22	14	21	20	23
		-A 2	4	7	17	11	18	13	16	17	14
		+A 2	4	7	15	14	13	10	12	12	10

0,400 = day-degrees; -A = no aldicarb +A = aldicarb treated

1 = first planting date 2 = second planting date

Fig.21 Field Trial 2: Cultivar, physiological age and percentage ground cover (aldicarb treated, first planting date)



degree seed tubers senesced earlier in the autumn than plants grown from 0 day-degree seed in Estima, Maris Piper and Maris Bard plots; in Pentland Dell plots physiologically old seed maintained a higher percentage ground cover than young seed throughout the growth period (Fig 21). The highest peak percentage ground cover was 79% for 0 day-degree aldicarb treated Maris Piper from the first planting date.

Mean PGCD for all plots was 19.9% and data for all treatments are summarised in Table 42. Maris Piper had the greatest PGCD followed by Estima, Maris Bard and Pentland Dell. All differences were significant ($\underline{P}< 0.10$) except between Maris Bard and Pentland Dell (Table 43 and Fig 22).

Physiological age had no significant overall effect on PGCD or when individual cultivars were considered (Appendix 5).

The first planting date had a significantly higher overall PGCD than the second ($\underline{P}< 0.01$, Table 44). When cultivars were considered individually all four cultivars had higher PGCD's from the first planting date but this was only significantly greater for Maris Piper ($\underline{P}< 0.10$, Table 45). The three weeks difference between planting dates enabled plants from the first date to emerge and produce foliage earlier than plants from the second planting date but the advantage of higher PGCD's gained by this was not reflected in terms of higher yields (Appendix 3).

Aldicarb treatment increased overall PGCD ($\underline{P}<0.01$, Table 46) but had no significant effect when cultivars were considered individually (Appendix 5). At the first planting date aldicarb treatment significantly increased PGCD ($\underline{P}< 0.01$, Table 47) but for the second planting date, three weeks later, there was no significant difference between treated and untreated plots. In soils that have had no previous application of aldicarb, Suett and Jukes (1988) reported a 50% loss of aldicarb residues (at 15C) in 25-50 days; in previously treated soils this loss

TABLE 42 Field Trial 2: Mean PGCD's for all treatments

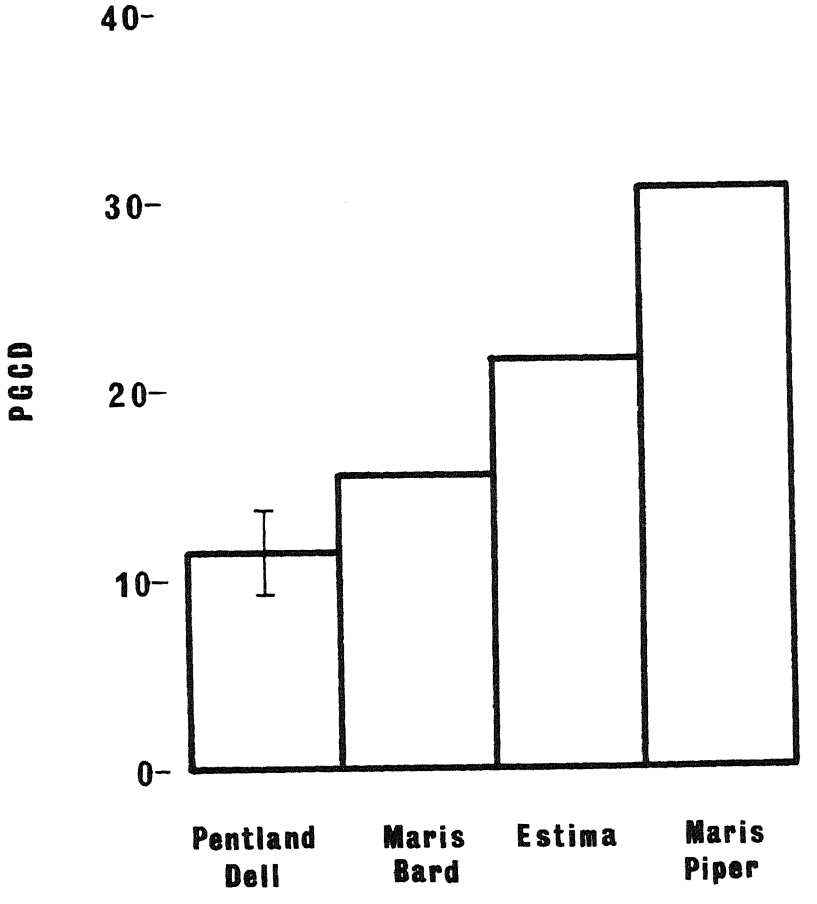
Day-degrees	0				400			
Planting date	1		2		1		2	
Aldicarb	-	+	-	+	-	+	-	+
Estima	16.8	24.4	25.2	21.2	19.1	30.7	16.6	19.5
M. Piper	37.7	44.0	20.5	18.8	30.4	40.8	27.2	26.9
M. Bard	21.1	18.0	13.9	11.8	13.7	16.6	16.3	13.5
P. Dell	14.2	13.5	10.3	7.1	8.9	20.0	10.1	8.4

S.E.D.(3 d.f.) 5.28

TABLE 43 Field Trial 2: Cultivar and PGCD

Estima	Maris Piper	Maris Bard	Pentland Dell	S.E.D.(3 d.f.)
21.7	30.8	15.6	11.6	2.43

Fig.22 Field Trial 2: Cultivar and PGCD



(Bar represents 95% confidence limits for all cultivars)

TABLE 44 Field Trial 2: Planting date and PGCD

	Planting date		
	1	2	S.E.D.(1 d.f.)
PGCD	23.1	16.7	1.72

TABLE 45 Field Trial 2: Cultivar, planting date and PGCD

	Planting date		
	1	2	S.E.D.(3 d.f.)
Estima	22.8	20.6	3.44
Maris Piper	38.2	23.3	
Maris Bard	17.4	13.9	
Pentland Dell	14.1	9.0	

TABLE 46 Field Trial 2: Aldicarb and PGCD

	Aldicarb		
	-	+	S.E.D.(1 d.f.)
PGCD	18.9	20.9	0.72

TABLE 47 Field Trial 2: Planting date, aldicarb and PGCD

		Aldicarb		
		-	+	S.E.D.(1 d.f.)
Planting date	1	20.2	26.0	1.87
	2	17.5	15.9	

occurred in only 5-17 days. The trial site has had several applications of aldicarb and used at recommended rates it now has little apparent effect upon crop growth and yield (W. Smith, pers. comm.). This suggests that previous treatment with aldicarb is causing the accelerated degradation of the chemical and its oxidation products, resulting in there being little effect on nematodes at the second planting date.

Growth Analysis Six Weeks after Planting

All cultivars had haulm, root and total plant fresh weights that were heavier 6 weeks after the second than the first planting date. The only exception was for Maris Bard where mean root fresh weight was greater from the first than the second date (Table 48). Higher soil and air temperatures at the second planting date resulted in faster growing plants with greater fresh weights six weeks after planting. The nematode population density in each root system was larger at the second than the first planting date (Table 48) because the faster growing, larger roots stimulated more nematodes to hatch and invade. From the first planting date Maris Bard plants had the highest haulm and total fresh weights followed by Maris Piper, Estima and Pentland Dell; Maris Bard also had the greatest root weight followed by Estima, Maris Piper and Pentland Dell. Maris Bard is an early maturing "first early" cultivar and fast initial growth rates are a characteristic of this maturity class. From the second planting date haulm, root and total plant fresh weights 6 weeks after planting decreased from Maris Piper, Estima and Maris Bard to Pentland Dell (Table 48).

Overall (Table 49) and when cultivars are considered individually (Table 50) plants grown from 400 day-degree seed tubers had significantly greater haulm, root and total plant fresh weights 6 weeks after the first planting date than those from 0 day-degree seed tubers ($P < 0.10$) but there were no

TABLE 48 Field Trial 2: Cultivar, planting date and growth analysis 6 weeks after planting

	Planting date	Estima	Maris Piper	Maris Bard	Pentland Dell	SED(3df)
Haulm weight(g)	1	26.7	33.1	33.9	18.8	3.81
	2	151.2	188.7	124.5	65.2	25.38
Root weight(g)	1	7.82	7.09	8.81	4.99	0.625
	2	9.55	10.15	7.91	6.43	1.311
Total plant weight(g)	1	39.9	46.0	51.5	28.5	4.57
	2	179.3	226.6	151.0	89.1	29.33
Total nems/ root system	1	8213	7397	7631	8221	267.1
	2	8697	8452	8703	8610	164.5

TABLE 49 Field Trial 2: Planting date, physiological age and growth analysis 6 weeks after planting

	Planting date	Day-degrees 0	Day-degrees 400	S.E.D.(1d.f.)
Haulm weight(g)	1	21.7	34.5	2.70
	2	134.8	130.0	17.94
Root weight(g)	1	6.32	8.03	0.442
	2	8.43	8.59	0.927
Total plant weight(g)	1	32.8	50.2	3.23
	2	166.5	168.7	20.74
Total nems/ root system	1	9654	10203	179.8
	2	8622	8613	116.3

TABLE 50 Field Trial 2: Cultivar, planting date, physiological age and growth analysis 6 weeks after planting

Day-degrees		Plant date	Estima	Maris Piper	Maris Bard	Pentland Dell	SED(3df)
0	Haulm wt(g)	1	21.2	27.5	21.6	16.6	5.39
		2	181.9	146.7	130.3	80.2	35.89
	Root wt(g)	1	6.58	5.20	8.63	4.88	0.883
		2	9.79	8.23	8.98	6.72	1.853
	Total plant wt(g)	1	32.1	37.4	36.5	25.1	6.46
		2	212.0	186.8	160.2	107.0	41.48
	Nems/ root system	1	10048	8871	9627	10072	359.5
		2	8546	8152	9033	8757	232.7
400	Haulm wt(g)	1	32.2	38.8	46.2	20.9	5.39
		2	120.5	230.6	118.6	50.1	35.89
	Root wt(g)	1	9.06	8.97	8.99	5.11	0.883
		2	9.30	12.08	6.85	6.14	1.853
	Total plant wt(g)	1	47.8	54.6	66.5	32.0	6.46
		2	146.7	266.3	141.7	71.2	41.48
	Nems/ root system	1	10784	9868	10145	10014	359.5
		2	8849	8753	8373	8463	232.7

significant differences 6 weeks after the second planting date. By 6 weeks after the second planting date the faster initial growth rates as a result of the physiological age of the seed are being compensated for by earlier senescence and so, overall, physiological age had little effect on plant size.

Aldicarb treatment had no significant effect on plant weight 6 weeks after either planting date (Appendix 6).

3.3.4 Conclusions

As in Field Trial 1, yields were low (mean ware yield 10.96t/ha) as a result of the damage to the plants' roots inflicted by a moderately high nematode population density (mean Pi = 347 eggs per g soil) inhibiting the uptake of water and nutrients, resulting in stunted canopies which could only intercept a limited amount of incident radiation (maximum PGCD = 44%).

There was no significant difference in Pi between Estima, Maris Piper and Maris Bard plots but Pentland Dell plots had a significantly higher Pi than the other cultivars. Overall nematode multiplication rates were low (mean Pf/Pi = 2.33) due to the density dependent nature of nematode multiplication.

Overall, Maris Piper produced the greatest yield followed by Maris Bard, Estima and Pentland Dell. The higher Pi in Pentland Dell plots would have contributed to its lower yield but the numbers of nematodes in the root systems 6 weeks after planting were not significantly different between cultivars. Maris Piper had the greatest PGCD followed by Estima, Maris Bard and Pentland Dell. Maris Piper was able to intercept more incident radiation and produce larger ware yields than the other cultivars; Pentland Dell intercepted the least radiation and had the lowest yields.

Seed tuber physiological age had no overall effect on PGCD's and yield. However, for Maris Bard, plants grown from 0 day-degree seed tubers outyielded those from 400. Physiological

ageing advanced Maris Bard's inherently early vegetative cycle to such an extent that early senescence reduced yield. For all cultivars except Pentland Dell plants grown from 400 day-degree seed tubers had larger canopies early in the growing season and senesced earlier than plants grown from 0 day-degree seed tubers; in Pentland Dell plots plants grown from physiologically old seed maintained larger canopies throughout the growing season.

Planting date had no significant effect on yield although all cultivars had higher PGCD's from the first planting date. The increase in intercepted solar radiation by the first planting date plots was not reflected in higher yields.

Aldicarb treatment had no effect on Pi, Pf or Pf/Pi for any cultivar. Treated plots had higher overall PGCD's for the first planting date but not the second. However, yield was not affected by aldicarb treatment at either planting date.

CHAPTER 4

FIELD TRIALS 3, 4 AND GENERAL DISCUSSION OF FIELD TRIALS 1 - 4

CHAPTER 4 FIELD TRIALS 3, 4 AND GENERAL DISCUSSION OF FIELD TRIALS 1-4

4.1 SITE PREPARATION FOR 1988 TRIALS

4.1.1 Strategy

The field trials of 1987 had shown aldicarb to have little effect in reducing pre-planting nematode population density. Where nematicides are not used there are four alternative methods of achieving a range of Pi's in field trials (Evans, 1982b). Each method has advantages and disadvatages but Evans concluded that if large differences in nutrient take off between treatments can be avoided then the preparatory treatment of the trial site in the previous year to produce a range of Pi's would give the best result.

This strategy was adopted to provide adjacent areas with low and high Pi's for the 1988 trials.

4.1.2 Methods

The preliminary Field No 1 nematode population density survey (Fig 9) was examined for adjacent areas of low and medium population density which avoided areas where Trials 1 and 2 were sited. Grids 1,2,3 and 8,9,10 were chosen, with mean Pi's of 65 and 194 eggs/g soil respectively.

In the spring of 1987 grids 1,2 and 10 were planted with Maris Piper to allow the population of G. pallida in these areas to increase during the growing season and provide areas of high Pi in 1988. Grids 1,2 and 10 were planted with the partially resistant cultivar Sante to reduce the multiplication of G. pallida and leave areas of low Pi for 1988. These areas were planted and harvested mechanically by staff at Rosedene Farm, who treated this area as a commercial crop.

This pre-treatment with a non resistant and partially resistant cultivar provided a rectangular trial site of 3750m2 (75 x 25m) for 1988.

4.1.3 Siting Field Trials 3 and 4

In January 1988 each pre-treated 25m2 block was divided into quarters and 20 cores of soil were removed from each in a systematic fashion. The population densities in each of the 24 quarters (12.5m2) are shown in Fig 23. Based on this data Field Trial 3 was sited within grids S1 to S4 (low Pi) and P9 to P12 (high Pi) and Field Trial 4 within grids S6,7,10,11 (low Pi) and P2,3,6,7 (high Pi).

4.2 FIELD TRIAL 3

The effect of seed tuber physiological age, planting date and initial G.pallida population density on the growth and yield of the first early cultivar Maris Bard, second early Estima and maincrop cultivars Maris Piper and Pentland Dell.

4.2.1 Experimental Design

Seed tubers of Maris Bard, Estima, Maris Piper and Pentland Dell were conditioned to two physiological ages (0 and 400 day-degrees above 4°C) and planted at two different dates into plots with both low and high Pi's.

The trial consisted of six blocks: three in the low and three in the high Pi areas. Within every block there were 16 plots, each containing one randomly assigned replicate of a treatment (Fig 24). A plot contained two inner harvest rows between two guard rows. There were seven plants in a row guarded at each end by two Desiree plants (Fig 25).

4.2.2 Methods

Physiological Ageing of Seed Tubers

Seed tubers weighing 55-65g were conditioned in the same way as those for Field Trials 1 and 2 except that the tubers were initially at 10°C (rather than 12°C) and before conditioning was completed the temperature was increased to 13°C.

FIG. 23 Globodera pallida population densities produced by preparation in 1987

P1 343	P2 265	P3 207	P4 181	S1 17	S2 47
P5 411	P6 294	P7 267	P8 297	S3 47	S4 26
S5 28	S6 48	S7 74	S8 74	P9 514	P10 361
S9 68	S10 82	S11 52	S12 122	P11 438	P12 334

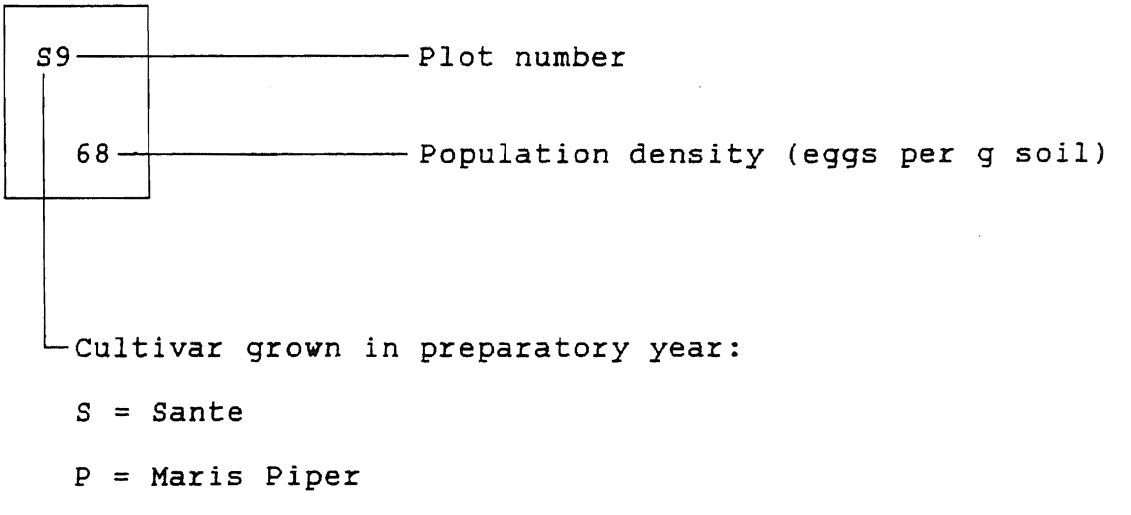


FIG. 24 Field Trial 3 : Experimental design

									Block
Low Pi	P 1 0	E 1 0	P 1 400	B 1 400	B 2 400	E 2 0	P 2 400	D 2 0	1
	B 1 0	P 2 0	E 1 400	D 1 400	D 1 0	B 2 0	D 2 400	E 2 400	
	D 1 400	B 1 400	B 2 0	E 1 0	P 1 0	P 2 0	P 1 400	B 2 400	2
	B 1 0	D 2 0	D 1 0	D 2 400	E 2 0	P 2 400	E 1 400	E 2 400	
	B 2 400	P 1 0	E 2 400	B 2 0	P 1 400	B 1 0	D 1 0	D 2 400	3
	E 1 400	E 1 0	P 2 0	D 2 0	E 2 0	D 1 400	P 2 400	B 1 400	
High Pi	P 1 400	D 1 0	B 2 400	P 1 0	E 1 400	B 1 0	B 1 400	E 1 0	1
	D 2 0	D 2 400	P 2 0	D 1 400	E 2 400	P 2 400	E 2 0	B 2 0	
	E 2 400	B 2 0	E 1 400	P 1 400	D 1 0	P 1 0	D 1 400	D 2 400	2
	B 2 400	P 2 400	B 1 400	E 2 0	D 2 0	E 1 0	B 1 0	P 2 0	
	E 1 400	B 1 0	P 2 400	E 2 400	D 2 400	B 1 400	P 1 400	B 2 400	3
	P 1 0	B 2 0	E 1 0	P 2 0	D 1 0	D 2 0	E 2 0	D 1 400	

P 1 — Planting date

0 — Day-degrees

Cultivar: P = Maris Piper
D = Pentland Dell
E = Estima
B = Maris Bard

FIG.25 Field Trial 3 : Plot design

D D D D

D D D D

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

D D D D

D D D D

Rows

G H H G

H = Harvest row

G = Guard row

X = Harvest plant

D = Desiree guard plant

G = Guard plant (same seed as harvest plants)

Fertiliser

The whole trial site received 420kg/ha of K2O and 200kg/ha of P2O5 in March and April respectively.

Planting

Seed tubers for the first planting date, 3-5 May, were placed by hand on the top of half formed ridges which were immediately reridged to cover the tubers with soil (Plates 6 and 7). For the second planting, 25-27 May, seed tubers were planted by hand at approximately 5cm depth into the fully formed ridges (Plate 8).

Weed and Disease Control

A pre-emergence spray of paraquat (Gramoxone 100; I.C.I.) at 1 litre (18% a.i.)/ha initially controlled all of the weeds that had emerged between planting and crop emergence. After crop emergence plots were hand weeded and rogued as required to prevent interspecific competition with the trial plants (Plate 8). Mancozeb and metalaxyl (Fubol 75; Ciba Geigy) at 2kg (67.5 and 7.5% a.i. respectively)/ha were applied in response to ADAS blight warnings; this was tank mixed with magnesium sulphate at 6kg/ha on 7 and 23 June.

Canopy Development

Percentage ground cover was measured at weekly intervals from 8 June until 7 September.

Soil Sampling

Pi and Pf were estimated from 30 cores of soil taken from each plot before planting and immediately after harvest (section 3.1.4).

Harvesting

On 23 September unsenesced haulms were desiccated with sulphuric acid at 170l/ha prior to hand harvesting from 26-29 September. Tubers were mechanically riddled over 10,20,30,40,50 and 60mm meshes; tubers greater than 40mm constituted ware yield.

Plate 6 Seed-tubers of Field Trials 3 (background) and 4 (foreground) placed on small ridges before being buried by mechanical ridging (4 May, 1988)

Plate 7 Mechanical ridging of seed-tubers in Field Trials 3 and 4 (5 May, 1988)

Plate 8 Field Trials 3 and 4

Second planting date of Trial 3 (26 May, 1988)

Trial 4 before weeding and rogueing (26 May, 1988)

4.2.3 Results and Discussion

Yield

Mean total, ware and percentage ware yield for each treatment are summarised in Table 51. Tuber yields from this trial were moderate with a mean ware yield of 19.1 t/ha accounting for 62.8% of the total yield of 27.4 t/ha.

Maris Piper had the greatest total and ware yield and Pentland Dell the least. Estima and Maris Bard were intermediate in yield but were not significantly different from each other (Table 52).

Overall, 0 day-degree plants outyielded 400 day-degree plants in both total and ware yield but this difference was only significant for total yield (Table 53). When cultivars are considered individually there were no significant differences in total yields (Table 54).

Plots with low Pi's had overall significantly greater total and ware yields than plots with high Pi's ($P < 0.01$, Table 55) and this was significant for every cultivar ($P < 0.10$, Table 56).

Total and ware yields from the second planting date were overall higher than from the first planting date ($P < 0.01$, Table 57). Considering the cultivars individually (Table 58), only in Estima plots was total yield significantly greater from the second planting date ($P < 0.10$). However, ware yield was significantly greater from the second planting date in Maris Piper, Estima and Maris Bard ($P < 0.05$). These effects were only found in plants grown from 400 day-degree seed tubers; within 0 day-degree plots, planting date had no significant effect on yield (Table 59).

Nematode Population Densities and Multiplication Rates

Mean Pi, Pf and Pf/Pi data for each treatment are summarised in Table 60). Overall mean Pi and Pf were 174 and 1000 eggs/g of soil, while the mean Pf/Pi ratio was 13.3.

TABLE 51 Field Trial 3: Mean yields for all treatments

			Cultivar							
			M Piper		P Dell		Estima		M Bard	
			Physiological age (day-degrees)							
Yield	Pi	Planting date	0	400	0	400	0	400	0	400
Total yield (t/ha)	L	1	45.9	38.2	38.3	31.3	36.7	27.5	36.2	36.2
		2	36.4	48.1	33.6	25.8	36.3	36.1	36.8	34.9
	H	1	28.7	24.8	12.4	9.7	14.6	6.7	15.7	13.8
		2	29.1	32.5	13.9	16.1	18.3	20.9	20.8	19.5
Ware yield (t/ha)	L	1	36.5	24.8	25.1	21.1	30.0	19.8	28.0	29.0
		2	28.2	39.0	20.8	15.4	30.2	29.7	30.6	29.2
	H	1	19.8	14.0	2.7	3.0	6.6	2.1	7.3	7.5
		2	20.9	25.3	2.0	6.9	11.7	13.8	14.8	15.9
Percentage ware	L	1	79.5	64.6	65.4	67.1	81.8	71.5	77.6	79.8
		2	77.6	81.0	60.4	57.5	83.3	82.3	83.3	83.9
	H	1	67.7	56.3	19.4	21.4	44.1	30.4	43.8	54.7
		2	71.6	77.9	11.7	40.9	57.5	63.5	70.3	81.4

S.E.D.(3 d.f.) Total yield = 3.73

Ware yield = 3.55

Percentage ware = 7.90

TABLE 52 Field Trial 3: Cultivar mean yields

	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Maris Piper	35.5	26.1	72.0
Pentland Dell	22.6	12.1	43.0
Estima	24.6	18.0	64.3
Maris Bard	26.7	20.3	71.8
S.E.D.(3 d.f.)	1.27	1.19	2.31

TABLE 53 Field Trial 3: Physiological age and yield

	Day-degrees		
	0	400	S.E.D.(1 d.f.)
Total yield (t/ha)	28.3	26.4	0.90
Ware yield (t/ha)	19.7	18.5	0.84
Percentage ware	62.2	63.4	1.64

TABLE 54 Field Trial 3: Physiological age,cultivar and yield

	Day-degrees	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Maris Piper	0	35.0	26.4	74.1
	400	35.9	25.8	70.0
Pentland Dell	0	24.5	12.6	39.2
	400	20.7	11.6	46.7
Estima	0	26.4	19.6	66.7
	400	22.8	16.3	61.9
Maris Bard	0	27.4	20.2	68.8
	400	26.1	20.4	74.9
S.E.D.(3 d.f.)		1.80	1.68	3.27

TABLE 55 Field Trial 3: Pi and yield

	Pi		
	Low	High	S.E.D.(1 d.f.)
Total yield (t/ha)	36.1	18.6	1.33
Ware yield (t/ha)	27.3	10.9	1.41
Percentage ware	74.8	50.8	2.31

TABLE 56 Field Trial 3: Cultivar, Pi and yield

	Pi	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Maris Piper	Low	42.1	32.1	75.7
	High	28.8	20.0	68.4
Pentland Dell	Low	32.3	20.6	62.6
	High	13.0	3.6	23.3
Estima	Low	34.1	27.4	79.7
	High	15.1	8.5	48.9
Maris Bard	Low	36.0	29.2	81.1
	High	17.4	11.3	62.5
S.E.D.(3 d.f.)		2.05	2.03	5.51

TABLE 57 Field Trial 3: Planting date and yield

	Planting date		
	1	2	S.E.D.(1 d.f.)
Total yield (t/ha)	26.0	28.7	0.90
Ware yield (t/ha)	17.3	20.9	0.84
Percentage ware	57.8	67.8	1.64

TABLE 58 Field Trial 3: Cultivar, planting date and yield

	Planting date	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Maris Piper	1	34.4	23.8	67.0
	2	36.5	28.4	77.1
Pentland Dell	1	22.9	12.9	43.3
	2	22.4	11.3	42.6
Estima	1	21.4	14.6	57.0
	2	27.9	21.3	71.6
Maris Bard	1	25.5	17.9	64.0
	2	28.0	22.6	79.7
S.E.D.(3 d.f.)		1.80	1.68	3.27

TABLE 59 Field Trial 3: Physiological age, planting date and yield

	Day-degrees				
	0		400		
Planting date	1	2	1	2	S.E.D.(1 d.f.)
Total yield (t/ha)	28.6	28.1	23.5	29.2	1.27
Ware yield (t/ha)	19.5	19.9	15.1	21.9	1.19
Percentage ware	59.9	64.5	55.7	71.1	2.31

TABLE 60 Field Trial 3: Mean Pi, Pf and Pf/Pi for all treatments

Day-degrees		0				400				
Planting date		1		2		1		2		
Pi		Low	High	Low	High	Low	High	Low	High	SED(3df)
Maris Piper	Pi	50	346	71	227	57	281	47	226	58.0
	Pf	828	1027	861	1031	655	934	747	1039	275.8
	Pf/Pi	26.6	3.0	54.6	4.6	15.7	3.7	38.8	4.9	15.04
Pentland Dell	Pi	65	373	62	325	74	261	77	259	58.0
	Pf	1386	975	1267	1522	1267	655	988	1119	275.8
	Pf/Pi	23.9	2.7	22.0	5.3	18.6	2.6	12.7	5.3	15.04
Estima	Pi	88	278	53	326	80	230	75	317	58.0
	Pf	852	1121	866	1323	1314	701	1159	1269	275.8
	Pf/Pi	14.7	4.2	21.3	5.0	29.6	3.5	15.6	4.4	15.04
Maris Bard	Pi	119	234	60	345	55	227	69	229	58.0
	Pf	1171	995	884	925	727	842	703	839	275.8
	Pf/Pi	10.0	4.5	16.2	2.9	24.5	3.9	15.2	3.7	15.04

Within the two prepared Pi levels, Pi was significantly greater in the high area than the low area ($P < 0.01$). Pf's were not significantly different but the overall Pf/Pi ratio was significantly greater in the low Pi area than the high area ($P < 0.10$, Table 61) due to the density dependent nature of PCN multiplication.

There was no significant difference in Pi between cultivars but the Pf's in Pentland Dell and Estima plots were significantly higher than those of Maris Piper and Maris Bard ($P < 0.05$) although they were not significantly different from each other (Table 62). Pf/Pi ratios were not significantly different between cultivars.

Plots with 400 day-degree plants overall had lower Pi's and Pf's than those with 0 day-degree plants ($P < 0.05$). Pf/Pi ratios were not significantly different between physiological ages (Table 63). Within individual cultivars the lower Pi's found in 400 day-degree plots were not significantly different from those of 0 day-degree plots (Table 64).

There were no significant overall differences in Pi, Pf or Pf/Pi between planting dates or when cultivars were considered individually (Appendix 7).

Plant Emergence and Canopy Development (Plate 9)

Percentage plant emergence data for each treatment on 8 June (11 and 35 days after planting dates 1 and 2 respectively) are shown in Table 65. Emergence from the first planting date is complete. For the second planting date emergence is higher in 400 day-degree plots than in 0 day-degree plots. Emergence is generally lower in the high than the low Pi areas.

Mean percentage ground covers for each treatment from June to September are summarised in Table 66). The data for Maris Bard are shown graphically in Fig 26. Data for all cultivars in first planting date low Pi plots are shown in Fig 27. In all cultivars,

TABLE 61 Field Trial 3: Pi treatment and Pi, Pf and Pf/Pi

	Pi		
	High	Low	S.E.D.(1 d.f.)
Pi	280	69	22.0
Pf	1020	980	138.8
Pf/Pi	4.0	22.6	7.54

TABLE 62 Field Trial 3: Cultivar and Pi, Pf and Pf/Pi

	Maris Piper	Pentland Dell	Estima	Maris Bard	S.E.D.(3 d.f.)
Pi	163	187	181	167	19.6
Pf	890	1147	1076	886	87.0
Pf/Pi	19.0	11.7	12.3	10.2	4.75

TABLE 63 Field Trial 3: Physiological age and Pi, Pf and Pf/Pi

	Day-degrees		
	0	400	S.E.D.(1 d.f.)
Pi	189	160	13.9
Pf	1065	935	61.5
Pf/Pi	13.8	12.7	3.36

TABLE 64 Field Trial 3: Cultivar, physiological age and Pi, Pf and Pf/Pi

	Day-degrees	Pi	Pf	Pf/Pi
Maris Piper	0	174	937	22.2
	400	153	844	15.8
Pentland Dell	0	206	1287	13.5
	400	168	1007	9.8
Estima	0	186	1041	11.3
	400	175	1111	13.3
Maris Bard	0	189	994	8.4
	400	145	778	12.1
S.E.D.(3 d.f.)		27.7	123.1	6.72

Plate 9 Field Trial 3 (23 June, 1988)

Estima (low Pi, 400 day-degrees). Foreground is the first planting date (70% ground cover), background is the second planting date (28% ground cover)

Poor growth in Pentland Dell plot (low Pi, 0 day-degrees, first planting date)

TABLE 65 Field Trial 3: Percentage plant emergence on 8 June (11 and 35 days after planting dates 1 and 2 respectively)

		Low Pi		High Pi	
		Planting date			
	Day-degrees	1	2	1	2
Maris Piper	0	98	10	95	0
	400	100	43	95	3
Pentland Dell	0	100	14	100	7
	400	100	69	100	33
Estima	0	100	19	100	2
	400	100	57	100	76
Maris Bard	0	100	38	100	19
	400	100	71	100	79

TABLE 66 Field Trial 3: Mean percentage ground covers for all treatments

				JUNE				JULY				AUGUST			SEPT
				8	16	23	30	8	14	21	28	4	14	30	7
				Days after planting date 1											
Treatment				35	43	50	57	65	71	78	85	92	102	118	126
MB	1	0	L	13	49	62	85	88	89	87	76	71	26	1	0
			H	11	27	44	53	44	53	45	17	11	2	0	0
	2	0	L	0	5	23	52	63	83	95	98	95	49	14	1
			H	0	2	22	30	34	59	55	67	70	38	3	2
	1	4	L	19	57	74	87	95	92	76	52	40	16	1	2
			H	14	32	37	54	37	34	32	7	4	1	0	0
	2	4	L	0	7	34	52	55	77	90	94	92	77	18	10
			H	0	4	24	35	43	48	47	48	59	19	2	0
PD	1	0	L	9	32	56	81	85	93	93	93	86	78	29	28
			H	6	17	37	46	36	41	37	30	36	26	4	4
	2	0	L	0	3	25	53	70	89	96	99	99	98	70	56
			H	0	2	15	33	37	59	59	47	67	47	13	10
	1	4	L	12	34	61	82	89	87	85	82	71	52	11	5
			H	13	26	40	50	31	28	27	15	14	4	1	0
	2	4	L	0	7	25	41	45	73	87	90	88	86	37	28
			H	0	2	17	30	36	47	52	48	67	53	25	15
E	1	0	L	5	23	44	77	89	97	95	94	88	59	10	7
			H	3	17	33	45	44	60	54	39	43	15	3	0
	2	0	L	0	5	20	47	75	94	100	100	97	88	44	19
			H	0	1	16	32	33	57	70	66	73	48	5	7
	1	4	L	13	44	70	83	88	81	66	44	32	9	1	0
			H	7	21	33	37	26	33	28	10	8	2	0	0
	2	4	L	0	4	28	49	66	90	98	97	94	85	47	27
			H	0	4	20	36	43	65	69	71	80	48	4	2
MP	1	0	L	4	21	45	72	93	95	100	98	99	95	75	73
			H	4	11	34	43	47	59	64	65	77	67	33	24
	2	0	L	0	1	20	51	64	83	95	100	97	93	61	61
			H	0	1	15	32	41	60	53	79	86	84	44	38
	1	4	L	15	46	76	85	87	92	96	92	90	75	44	38
			H	8	22	45	57	60	70	74	70	76	60	18	12
	2	4	L	0	6	33	52	75	95	99	98	95	90	56	41
			H	0	3	21	35	45	68	79	90	91	83	52	40

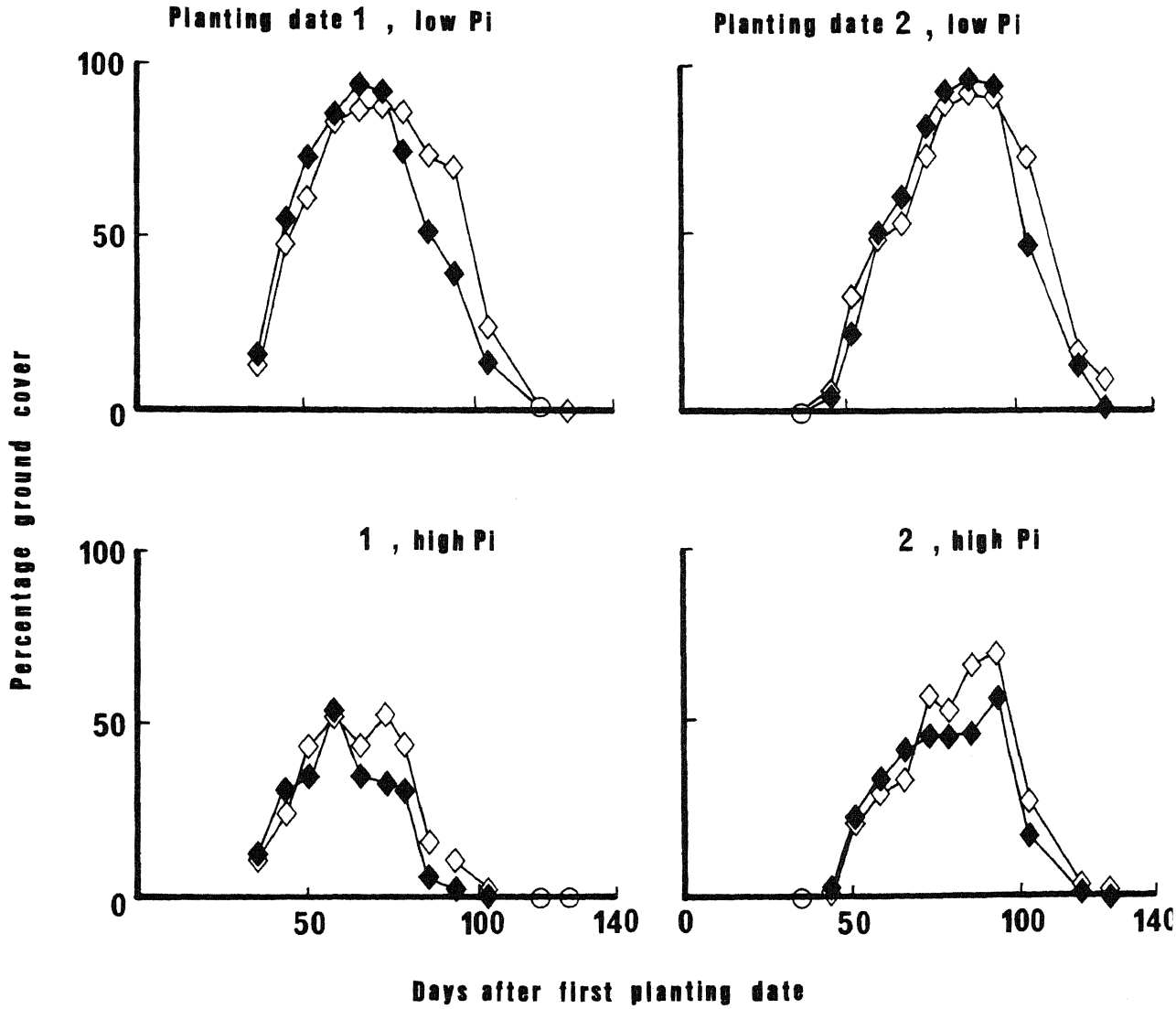
MB = Maris Bard, PD = Pentland Dell, E = Estima, MP = Maris Piper

1 = Planting date 1, 2 = Planting date 2

0 = 0 day-degrees, 4 = 400 day-degrees

L = Low Pi, H = High Pi

Fig.26 Field Trial 3: Percentage ground covers for Maris Bard plots

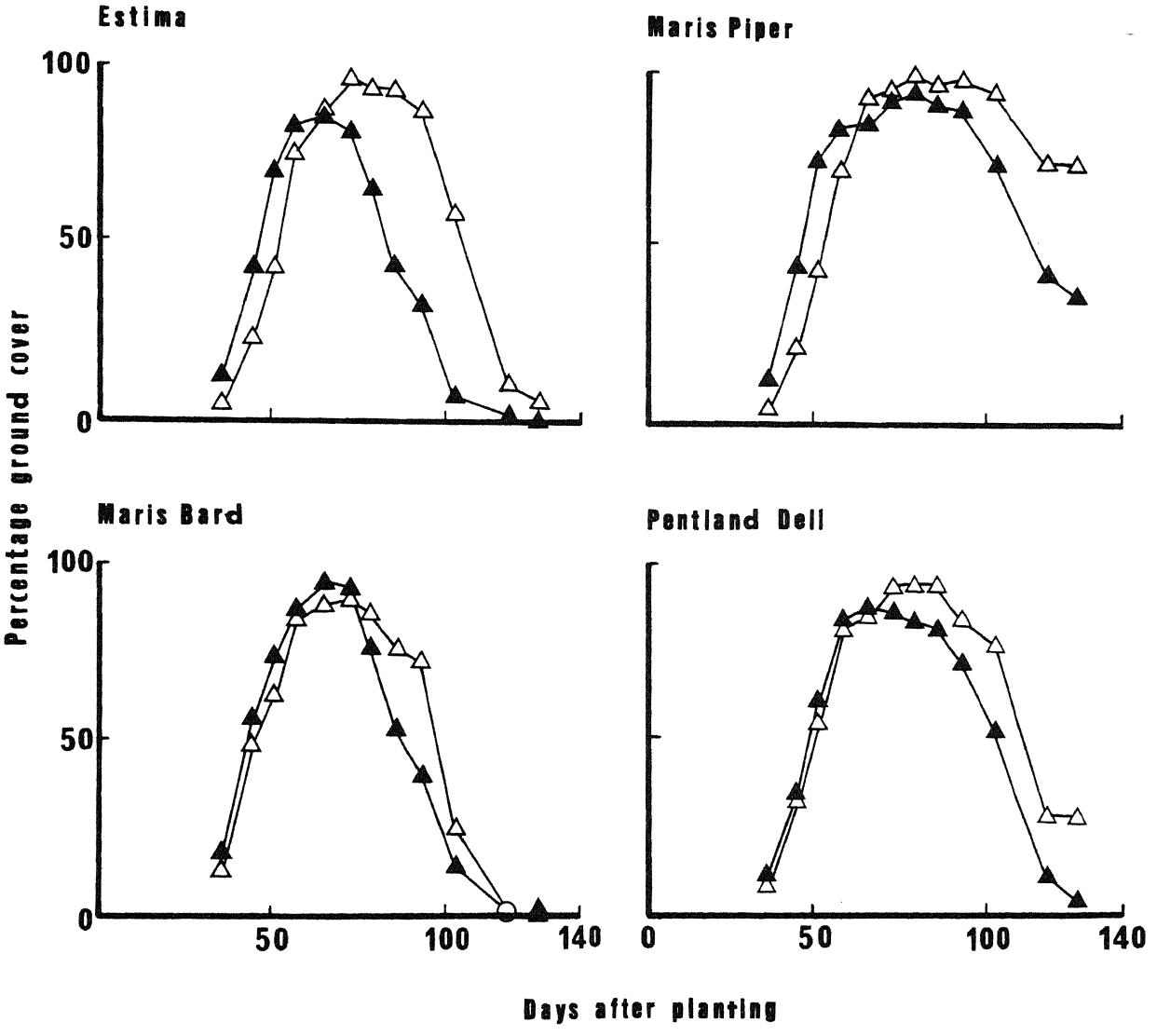


◇ = 0 day-degrees

◆ = 400 day-degrees

○ = equal data values

Fig.27 Field Trial 3: Cultivar, physiological age and percentage ground cover



△ = 0 day-degrees

▲ = 400 day-degrees

○ = equal data values

plants grown from physiologically old seed had larger canopies early in the growing season than plants grown from physiologically young seed. At 50-70 days after planting date 1, there is little difference in percentage ground cover between physiological ages within a cultivar. For all cultivars, plants grown from 400 day-degree seed tubers senesced earlier than plants grown from 0 day-degree seed tubers.

Mean PGCD for all plots was 37.1 and data for all treatments are summarised in Table 67. Maris Piper had the greatest PGCD followed by Pentland Dell, Maris Bard and Estima. All differences were significant ($P < 0.10$) except between Maris Bard and Estima (Table 68).

Both physiological age and planting date had no significant overall effect on PGCD or when cultivars are considered individually (Appendix 8).

Mean PGCD was significantly greater in the low Pi area than the high area ($P < 0.01$, Table 69) as was the case when each cultivar was considered individually (Table 70). The devastating effect of a high Pi on the canopy growth of Maris Bard can be seen in Fig 26.

4.2.4 Conclusions

Yields from this field trial were moderate with a mean ware yield of 19.1t/ha from an overall mean Pi of 174 eggs per g of soil. Earlier planting would have allowed more solar radiation to be intercepted and potential yields to be higher (Fig. 28 and Appendix 2). The strategy of growing a non resistant and a partially resistant cultivar on the trial site in the previous year to produce areas of high and low Pi for this trial was successful; the high Pi areas had a mean Pi of 280 eggs per g soil and the low 69. Mean Pf/Pi's in the high and low areas were 4.0 and 22.6 respectively, demonstrating the density dependent nature of nematode multiplication. There were no significant

TABLE 67 Field Trial 3: Mean PGCD's for all treatments

		Planting date			
		1		2	
		Day-degrees			
	Pi	0	400	0	400
Maris Piper	Low	58.2	56.7	43.2	50.8
	High	29.9	39.1	26.3	34.2
Pentland Dell	Low	47.0	51.3	45.4	45.4
	High	27.4	27.3	28.9	30.9
Estima	Low	49.5	43.7	48.3	44.9
	High	20.3	14.0	29.7	26.3
Maris Bard	Low	46.0	42.1	40.2	42.0
	High	26.3	19.3	27.6	31.5

S.E.D.(3 d.f.) 5.67

TABLE 68 Field Trial 3: PGCD and cultivar

	Maris Piper	Pentland Dell	Estima	Maris Bard	S.E.D.(3 d.f.)
PGCD	42.3	38.0	34.0	34.4	1.95

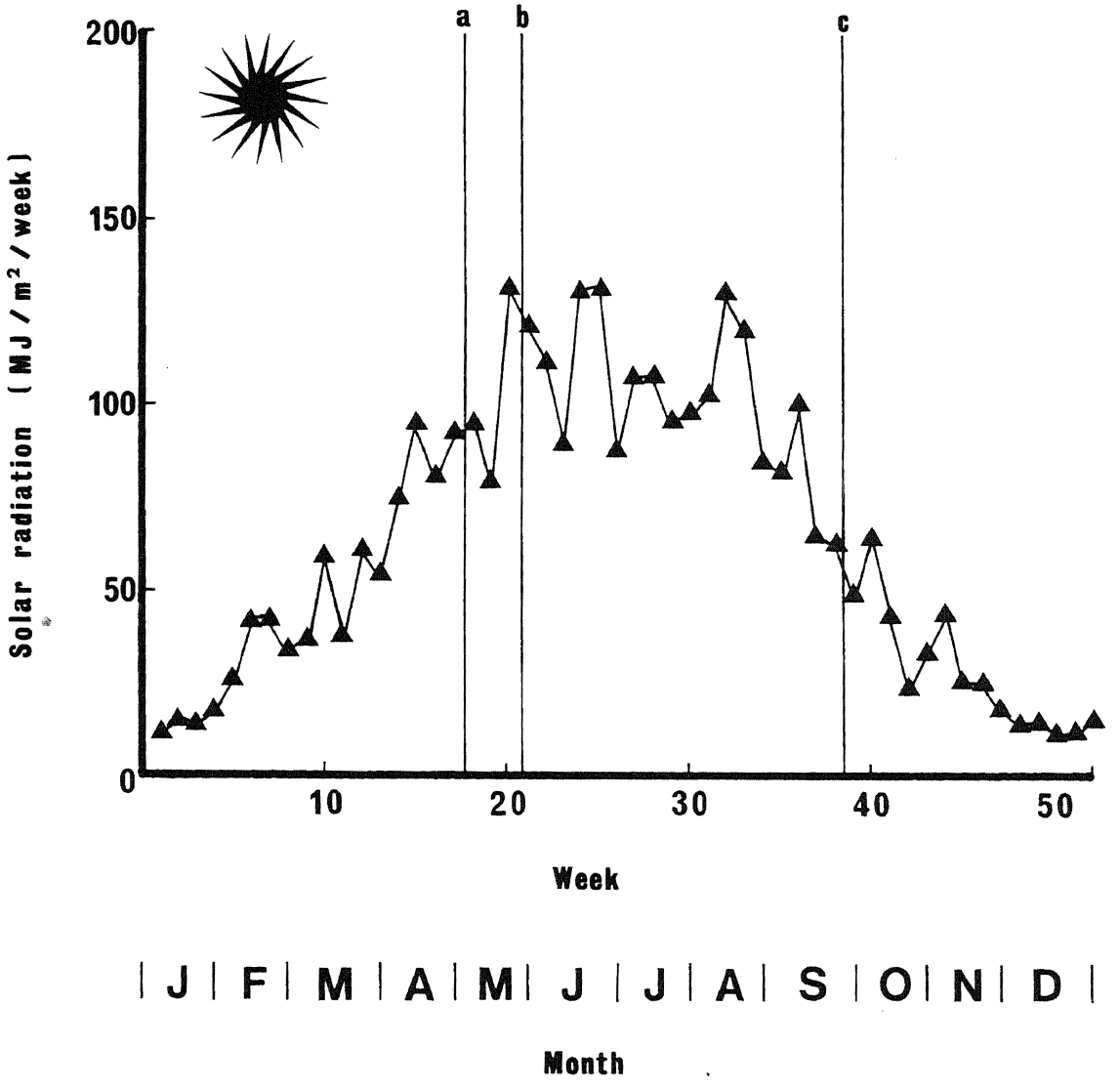
TABLE 69 Field Trial 3: Pi and PGCD

	Pi		
	Low	High	S.E.D.(1 d.f.)
PGCD	27.1	47.2	1.90

TABLE 70 Field Trial 3: Cultivar, Pi and PGCD

	Pi		
	Low	High	S.E.D.(3 d.f.)
Maris Piper	52.2	32.4	3.05
Pentland Dell	47.3	28.6	
Estima	46.6	21.3	
Maris Bard	42.6	26.2	

Fig.28 Incident solar radiation 1988 (Broom's Barn)



a = Field Trial 4 and Field Trial 3, planting date 1

b = Field Trial 3, planting date 2

c = Harvest

differences in Pf/Pi ratios between cultivars. In low Pi areas PGCD's and yields were larger for each cultivar than in the high Pi areas, the lower population densities of nematodes having a less damaging effect on the growth of the plants.

Maris Piper had the greatest total and ware yields followed by Estima, Maris Bard and Pentland Dell, although Estima and Maris Bard were not significantly different. Maris Piper also had the highest PGCD followed by Pentland Dell, Maris Bard and Estima but the relatively high PGCD of Pentland Dell was not transformed into yield.

Overall, 0 day-degree plots outyielded 400 day-degree plots but this was not significant for any individual cultivar. Physiological age had no significant effect on PGCD or ware yield in any cultivar. However, in all cultivars plants grown from 400 day-degree seed tubers produced larger canopies early in the growing season than those grown from 0 day-degree seed but, by 50-70 days after planting, physiological age had little effect on canopy size. Plants grown from 400 day-degree seed tubers senesced earlier in the autumn than those grown from unconditioned seed tubers.

Surprisingly, overall yield from the second planting date was greater than from the first. The only significant differences were in 400 day-degree plots where for Maris Piper, Estima and Maris Bard the ware yields were greater from the later planting date.

4.3 FIELD TRIAL 4

The effect of seed tuber physiological age and initial G. pallida population density on the growth and yield of the partially resistant cultivars Morag and Sante and clone 12288 and the non resistant cultivars Maris Piper and Pentland Dell.

4.3.1 Experimental Design

Seed tubers of Morag, Sante, Maris Piper, Pentland Dell and clone 12288 were conditioned to two physiological ages (0 and 400 day-degrees above 4℃) and planted in plots with both low and high Pi's.

The trial consisted of six blocks: three in the low and three in the high Pi areas. Within every block there were eight plots each containing one randomly assigned replicate of a treatment (Fig 29). A plot contained two inner harvest rows between two guard rows. There were 12 plants in a row guarded at each end by two Desiree plants (Fig 30).

4.3.2 Methods

Physiological ageing of seed tubers, fertiliser, planting (3-5 May only), weed and disease control (Plate 8), measurement of canopy development, soil sampling and harvesting were carried out in the same way as for Field Trial 3.

Growth Analysis

On 7 July, two plants were carefully harvested from each plot, dissected and a growth analysis carried out as for plants from Field Trial 1 (section 3.2.2). A 2g root sub-sample was preserved in FAA.

4.3.3 Results and Discussion

Yield

Mean total, ware and percentage ware yield for each treatment are summarised in Table 71. Tuber yields from this trial were reasonable with a mean ware yield of 26.8t/ha

FIG. 29 Field Trial 4 : Experimental design

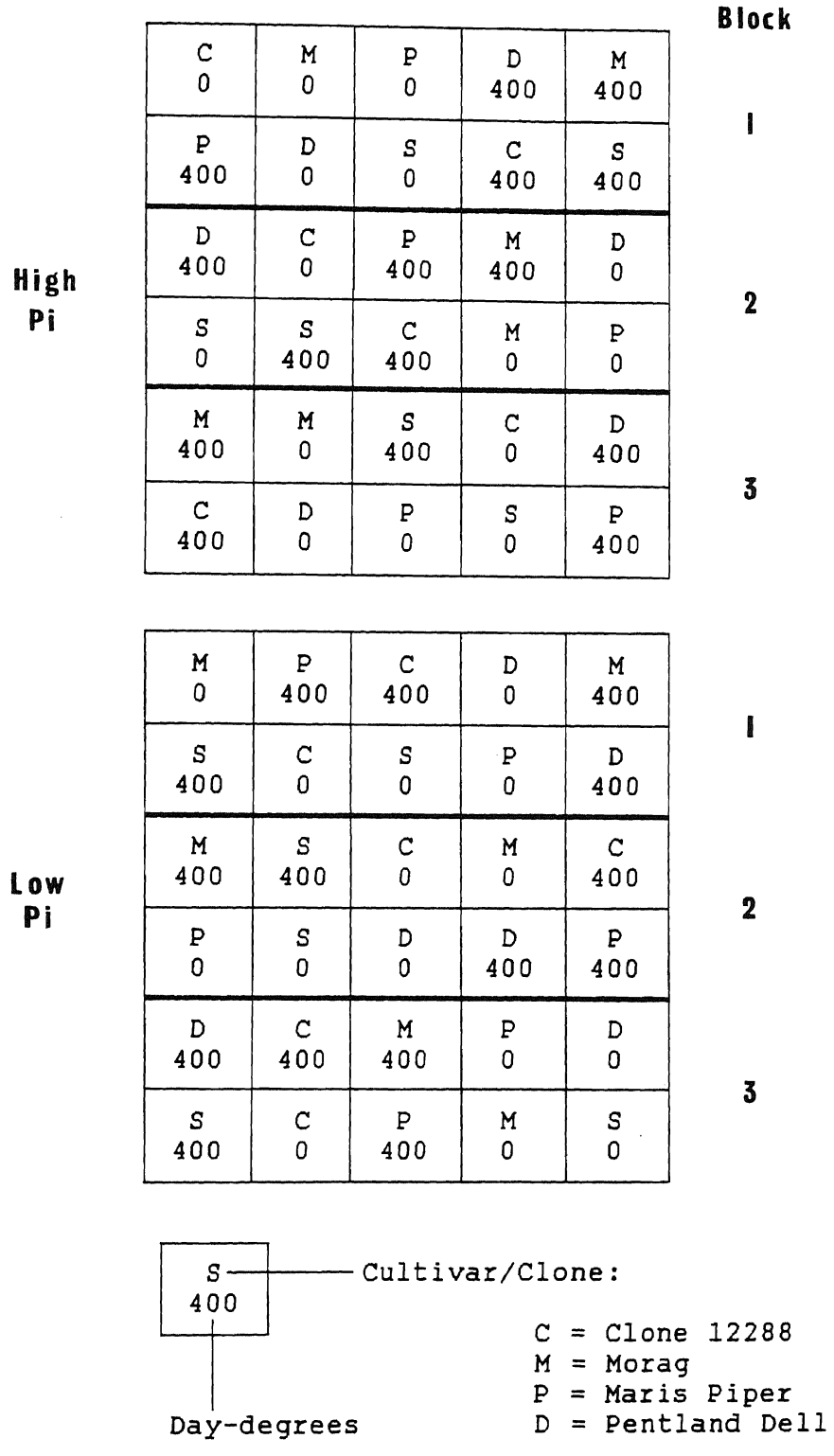


FIG. 30 Field Trial 4 : Plot design

D D D D

D D D D

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

G X X G

D D D D

D D D D

Rows

G H H G

H = Harvest row

G = Guard row

X = Harvest plant

D = Desiree guard plant

G = Guard plant (same seed as harvest plants)

TABLE 71 Field Trial 4: Mean yields for all treatments

		Total yield (t/ha)		Ware yield (t/ha)		Percentage ware	
		Low	High	Low	High	Low	High
Clone 12288	0	44.23	42.23	41.97	40.26	44.67	95.44
	400	34.83	37.20	29.17	34.37	82.25	92.38
Morag	0	39.70	39.33	33.88	33.02	84.58	82.12
	400	33.48	32.52	24.15	23.06	72.12	70.44
Sante	0	40.46	40.73	33.89	36.36	83.81	89.18
	400	41.93	34.76	33.72	28.23	80.30	79.93
Maris Piper	0	36.90	35.24	26.69	28.12	71.93	79.26
	400	30.86	36.72	20.48	25.51	58.93	69.52
Pentland Dell	0	26.00	23.56	13.32	11.04	45.84	44.95
	400	18.55	23.24	6.09	13.16	31.83	49.53

S.E.D. not available

accounting for 73.0% of the total yield of 34.6t/ha.

Sante had the highest total yield followed by clone 12288, Morag, Maris Piper and Pentland Dell. There were no significant differences in total yield between clone 12288, Morag and Maris Piper but Pentland Dell had a significantly lower yield than the other cultivars/clone. Clone 12288 had the highest ware yield followed by Sante, Morag, Maris Piper and Pentland Dell. The ware yield of clone 12288 was significantly greater than Morag, Maris Piper and Pentland Dell ($P< 0.05$) but not Sante. Clone 12288 had a very high percentage ware of 91%, which was significantly greater than those of the other cultivars ($P< 0.05$) contributing to its large ware yield (Table 72).

Overall, 0 day-degree plants outyielded 400 day-degree plants in both total and ware yield ($P< 0.05$ and $P< 0.01$ respectively, Table 73). When the cultivars/clone are considered individually the ware yield from 0 day-degree plants exceeded that from 400 day-degree plants in each case but this was not significant for any individual cultivar (Table 74). If the ware yield of each cultivar/clone is expressed as a percentage of the tolerant cultivar Maris Piper (Table 75) it can be seen that all cultivars/clone with partial resistance to *G. pallida* are more tolerant of nematode attack than Maris Piper while Pentland Dell is less tolerant.

There was little difference in overall total and ware yield between low and high Pi treatments (Table 76). Yields were also similar between Pi treatments when cultivars/clone were considered individually (Table 77). This is surprising when models relating yield loss to initial PCN density are considered (Seinhorst 1965, 1982; Brown, 1969, 1983; Brown and Sykes, 1983). Brown (1969), using data from a series of field trials, concluded that in U.K. field conditions each Pi increment of 20 eggs/g of soil caused a loss of yield of approximately 2 t/ha; Brown and

TABLE 72 Field Trial 4: Cultivar/clone mean yields

	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Clone 12288	36.95	36.44	91.18
Morag	36.26	28.53	77.31
Sante	39.47	33.05	83.31
Maris Piper	34.93	25.20	69.91
Pentland Dell	22.84	10.90	43.04
S.E.D.(4 d.f.)	3.199	3.242	4.525

TABLE 73 Field Trial 4: Physiological age and yield

	Day-degrees 0	400	S.E.D.(1 d.f.)
Total yield (t/ha)	36.85	32.41	2.023
Ware yield (t/ha)	29.86	23.80	2.051
Percentage ware	77.18	68.72	2.862

TABLE 74 Field Trial 4: Physiological age, cultivar/clone and yield

Cultivar	Day-degrees	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Clone 12288	0	43.28	41.12	95.05
	400	36.01	31.77	87.32
Morag	0	39.51	33.45	83.35
	400	33.00	23.61	71.28
Sante	0	40.60	35.12	86.50
	400	38.35	30.98	80.11
Maris Piper	0	36.07	27.40	75.59
	400	33.79	23.00	64.23
Pentland Dell	0	24.78	12.18	45.39
	400	20.89	9.63	40.68
S.E.D.(4 d.f.)		4.524	4.586	6.400

TABLE 75 Field Trial 4: Ware yield as a percentage of M. Piper

	Day-degrees	
	0	400
Maris Piper	100	100
Pentland Dell	44	42
Clone 12288	150	138
Morag	122	103
Sante	128	135

TABLE 76 Field Trial 4: Pi and yield

	Pi	
	Low	High
Total yield (t/ha)	34.70	34.55
Ware yield (t/ha)	26.34	27.31
Percentage ware	70.63	75.27

TABLE 77 Field Trial 4: Cultivar, Pi and yield

Cultivar	Pi	Total yield (t/ha)	Ware yield (t/ha)	Percentage ware
Clone 12288	Low	39.58	35.57	88.46
	High	39.72	37.32	93.91
Morag	Low	36.59	29.02	78.35
	High	35.92	28.04	76.28
Sante	Low	41.20	33.81	82.06
	High	37.74	32.29	84.55
Maris Piper	Low	33.88	23.58	65.43
	High	35.98	26.82	79.39
Pentland Dell	Low	22.28	9.71	38.83
	High	23.40	12.10	47.24

Sykes (1983) found average yield losses of 6.2 t/ha for each 20 eggs/g increment. The effect of Pi upon yield loss does vary between sites as reported by Brown (1983) who found that at different sites the effect of an increase in Pi of 20 eggs/g soil ranged from a decrease of 16.6 t/ha to a slight increase in yield.

Nematode Population Densities and Multiplication Rates

Mean Pi, Pf and Pf/Pi data for each treatment are summarised in Table 78.

Within the two prepared Pi levels, Pi and Pf were greater in the high than the low Pi area; the Pf/Pi ratio was larger in the low Pi than the high Pi area (Table 79). When individual cultivars/clone are considered the trends in Pi, Pf and Pf/Pi were similar in low and high Pi areas (Table 80).

There was no significant difference in Pi between cultivars/clone. The Pf's and Pf/Pi's of Maris Piper and Pentland Dell plots were significantly greater than those of clone 12288, Morag and Sante ($P < 0.05$). Pentland Dell had a significantly higher Pf (but not Pf/Pi) than Maris Piper; clone 12288, Morag and Sante were not significantly different from each other (Table 81).

Overall, plots with 400 day-degree seed tubers had the same mean Pi as those with 0 day-degrees. However, the Pf and Pf/Pi ratios from 400 day-degree plots were significantly lower than from 0 day-degree plots ($P < 0.01$, Table 82). Physiological age had no effect on Pf and Pf/Pi ratios for clone 12288, Morag or Sante. In Maris Piper and Pentland Dell plots, Pf was lower in 400 than 0 day-degree plots; Pf/Pi ratios were significantly lower in 400 day-degree plots for both cultivars ($P < 0.10$, Table 83).

Canopy Development

Mean percentage ground covers for each treatment from June

TABLE 78 Field Trial 4: Mean Pi, Pf and Pf/Pi for all treatments

Cultivar	Day-degrees	Pi		Pf		Pf/Pi	
		Low Pi	High Pi	Low Pi	High Pi	Low Pi	High Pi
Clone 12288	0	107	275	157	233	1.46	0.99
	400	116	353	148	339	1.36	0.98
Morag	0	117	310	246	270	2.38	1.06
	400	119	256	255	227	2.40	1.04
Sante	0	115	351	213	266	1.86	0.78
	400	98	273	248	276	3.01	1.00
Maris Piper	0	123	286	1013	1121	9.55	6.45
	400	140	351	745	1043	5.54	3.06
Pentland Dell	0	90	347	1220	1609	14.23	4.80
	400	126	285	875	928	7.31	3.42

TABLE 79 Field Trial 4: Pi treatment and Pi, Pf and Pf/Pi

	Pi		
	Low	High	S.E.D.
Pi	115	309	not available
Pf	512	631	
Pf/Pi	4.91	2.36	

TABLE 80 Field Trial 4: Cultivar, Pi treatment and Pi, Pf, Pf/Pi

	Clone 12288		Morag		Sante		M. Piper		P. Dell	
Pi	Low	High	Low	High	Low	High	Low	High	Low	High
Pi	112	314	118	283	106	312	132	318	108	316
Pf	152	286	250	249	230	271	879	1082	1048	1269
Pf/Pi	1.41	0.98	2.39	1.05	2.43	0.89	7.55	4.75	10.77	4.11

TABLE 81 Field Trial 4: Cultivar and Pi, Pf and Pf/Pi

Cultivar	Clone 12288	Morag	Sante	M.Piper	P.Dell	S.E.D.(4 d.f.)
Pi	213	200	209	225	212	36.2
Pf	219	250	251	980	1158	71.9
Pf/Pi	1.20	1.72	1.66	6.15	7.44	1.025

TABLE 82 Field Trial 4: Physiological age and Pi, Pf and Pf/Pi

Day-degrees	0	400	S.E.D.(1 d.f.)
Pi	212	212	22.9
Pf	635	508	45.5
Pf/Pi	4.36	2.91	0.648

TABLE 83 Field Trial 4; Cultivar, physiological age and Pi, Pf and Pf/Pi

Cultivar	Clone 12288		Morag		Sante		M.Piper		P.Dell		S.E.D.
Day-degrees	0	400	0	400	0	400	0	400	0	400	(4df)
Pi	191	234	214	188	233	186	205	245	218	206	51.1
Pf	195	244	258	241	239	262	1067	894	1415	902	101.7
Pf/Pi	1.23	1.17	1.72	1.72	1.32	2.00	8.00	4.30	9.51	5.37	1.450

to September are summarised in Table 84. Data from the low Pi area are shown graphically in Fig. 31.

In clone 12288, Morag and Sante plots plants grown from physiologically old seed tubers had larger canopies early in the growing season than plants from young seed but in Maris Piper and Pentland Dell plots there was little difference.

For all cultivars/clone, plants grown from 0 day-degree seed tubers had higher peak percentage ground covers and senesced later than plants grown from 400 day-degree seed tubers (Fig. 31).

Mean percentage ground cover duration (PGCD) for all plots was 49.5% and data for all treatments are summarised in Table 85. Maris Piper had the largest PGCD followed by Sante, clone 12288, Morag and Pentland Dell. The PGCD of Maris Piper was significantly higher and that of Pentland Dell significantly lower than the other cultivars/clone ($\underline{P}< 0.01$); clone 12288, Morag and Sante were not significantly different from each other (Table 86).

Overall, plants grown from 400 day-degree seed tubers had significantly lower PGCD's than those from 0 day-degree seed tubers ($\underline{P}< 0.01$, Table 87) but there were no significant differences when cultivars were considered individually (Table 88).

Plant Dry Weight

Mean plant dry weight data for each treatment on 7 July are summarised in Table 89. Plants were heavier overall in the low than in the high Pi area (Table 90), as was the case when cultivars/clone were considered individually (Table 91).

Sante had the highest dry weights followed by Morag, Pentland Dell, Maris Piper and clone 12288 (Table 92) but these differences were not significant.

Overall, plants grown from 400 day-degree seed tubers were

TABLE 84 Field Trial 4: Percentage ground covers for all treatments

			JUNE				JULY				AUGUST			SEPT
			8	16	23	30	8	14	21	28	4	14	30	7
			Days after planting date 1											
Treatment			35	43	50	57	65	71	78	85	92	102	118	126
12288	0	H	12	41	61	78	91	94	96	90	79	20	2	1
		L	10	42	69	85	96	99	100	98	90	25	0	0
	400	H	17	55	75	84	93	96	91	59	45	10	2	0
		L	17	52	72	84	88	93	91	53	54	15	3	2
Morag	0	H	8	32	49	55	78	84	80	81	74	49	27	14
		L	12	42	55	75	87	91	96	93	80	62	13	4
	400	H	11	35	57	52	69	78	82	59	45	28	9	5
		L	14	51	68	75	85	89	89	70	61	21	6	1
Sante	0	H	9	41	59	74	86	91	94	94	92	52	9	5
		L	14	41	65	84	95	96	99	98	97	61	1	0
	400	H	14	42	61	72	82	86	87	75	65	28	8	3
		L	21	53	80	93	98	99	99	88	77	27	4	1
M.Piper	0	H	8	28	48	70	84	92	91	92	88	68	44	24
		L	7	39	64	83	92	97	99	96	93	76	39	21
	400	H	12	37	60	70	91	94	96	94	92	65	45	24
		L	15	40	64	76	87	91	94	78	83	47	20	10
P.Dell	0	H	7	31	48	57	77	81	81	76	64	41	17	7
		L	12	38	55	79	78	85	83	75	73	42	11	2
	400	H	13	38	49	55	58	57	53	38	43	23	7	6
		L	16	36	64	68	68	59	62	36	33	9	2	0

0 = 0 day-degrees, 400 = 400 day-degrees

H = High Pi, L = Low Pi

Fig.31 Field Trial 4: Cultivar, physiological age and percentage ground cover (low Pi plots)

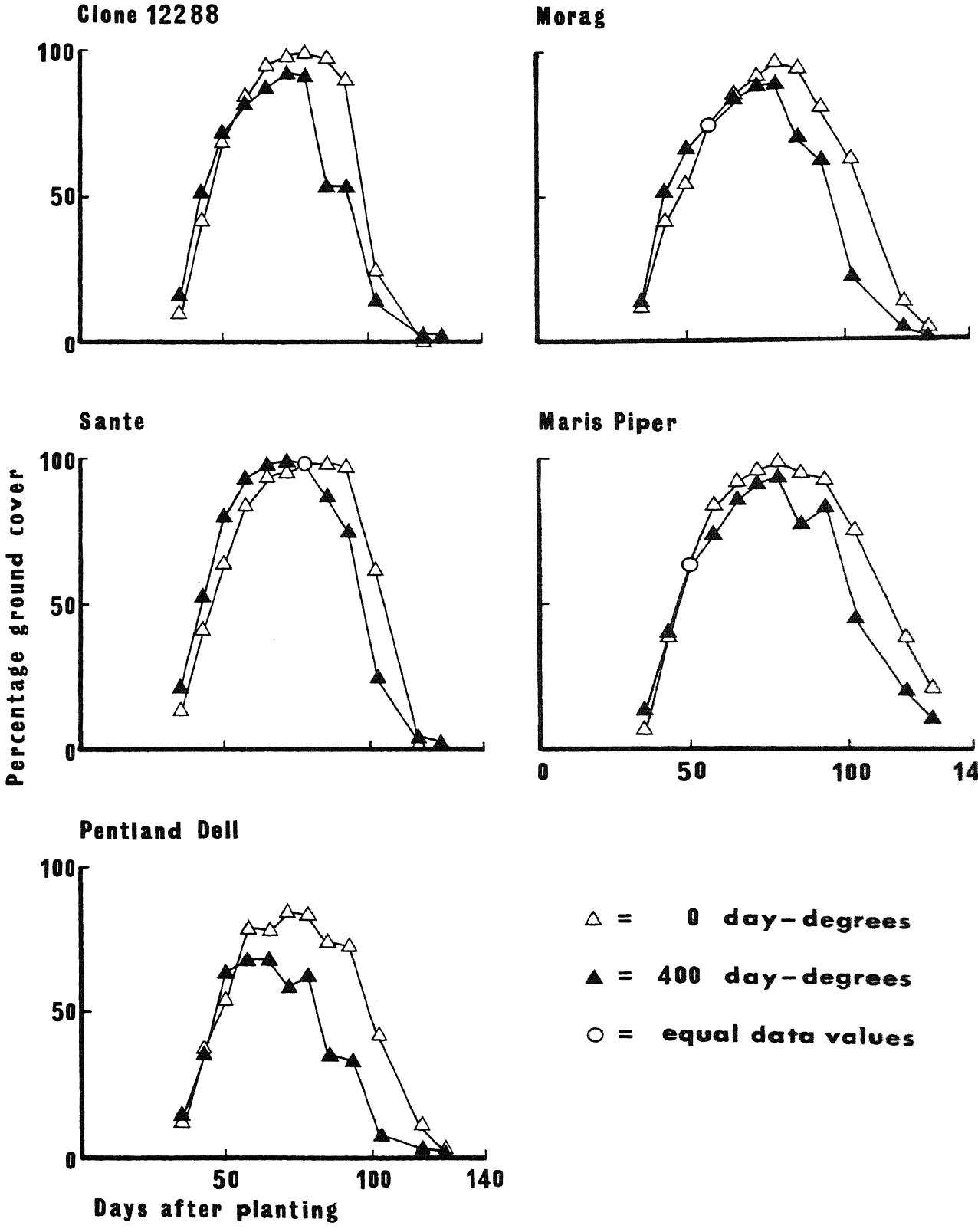


TABLE 85 Field Trial 4: Mean PGCD's for all treatments

		Pi	
		Low	High
Clone 12288	0	52.6	48.7
	400	45.1	45.0
Morag	0	54.7	50.2
	400	46.4	39.7
Sante	0	57.4	54.2
	400	54.7	46.2
M.Piper	0	63.6	58.7
	400	53.6	61.2
P.Dell	0	48.1	45.6
	400	32.3	32.6

S.E.D.(4 d.f.) 6.92

TABLE 86 Field Trial 4: Cultivar and PGCD

Clone 12288	Morag	Sante	Maris Piper	Pentland Dell	S.E.D.(4 d.f.)
47.8	47.8	53.1	59.3	39.6	6.92

TABLE 87 Field Trial 4: Physiological age and PGCD

Day-degrees		
0	400	S.E.D.(1 d.f.)
53.4	45.7	2.19

TABLE 88 Field Trial 4: Cultivar, physiological age and PGCD

	Day-degrees	
	0	400
Clone 12288	50.6	45.0
Morag	52.5	43.0
Sante	55.8	50.5
Maris Piper	61.1	57.4
Pentland Dell	46.8	32.4

S.E.D.(4 d.f.) 4.89

TABLE 89 Field Trial 4: Mean plant dry weight (g) on 7 July for all treatments

Day-degrees	0		400	
Pi	Low	High	Low	High
Clone 12288	71.4	52.1	86.8	90.9
Morag	85.4	76.4	102.1	76.2
Sante	88.4	82.8	115.8	86.9
Maris Piper	90.3	55.4	89.4	93.6
Pentland Dell	80.6	59.2	104.4	90.6

S.E.D.(4 d.f.) 18.07

TABLE 90 Field Trial 4: Pi and plant dry weight on 7 July

	Pi	
	Low	High
Plant dry weight (g)	91.5	76.4

TABLE 91 Field Trial 4: Cultivar, Pi and plant dry weight on 7 July

	Pi	
	Low	High
Clone 12288	79.1	71.5
Morag	93.8	76.3
Sante	102.1	84.8
Maris Piper	89.8	74.5
Pentland Dell	92.5	74.9

TABLE 92 Field Trial 4: Cultivar and plant dry weight on 7 July

	Plant dry weight (g)
Clone 12288	75.3
Morag	85.0
Sante	93.5
Maris Piper	82.2
Pentland Dell	83.7
S.E.D.(4 d.f.)	9.04

significantly heavier than those from 0 day-degree seed tubers ($P < 0.01$, Table 93) but when cultivars are considered individually there were no significant differences (Table 94).

4.3.4 Conclusions

The prepared low and high nematode population density areas had Pi's of 115 and 309 eggs per g soil respectively. Within each Pi level there were no significant differences in Pi between cultivars/clone and therefore all cultivars/clone faced a similar challenge.

Overall ware yields were reasonable at 36.4, 33.1, 28.5, 25.2 and 10.9 t/ha for clone 12288, Sante, Morag, Maris Piper and Pentland Dell respectively. PGCD was greatest for Maris Piper followed by Sante, clone 12288, Morag and Pentland Dell; the high yield of Maris Piper promised by its PGCD was not realised. There was, surprisingly, little difference in overall yields between the low and high Pi areas.

Six weeks after planting Sante plants were heaviest and clone 12288 lightest; Morag, Pentland Dell and Maris Piper were intermediate. Plant size at this time gave no indication of final yield with clone 12288 having the smallest plants but, eventually, the largest ware yield. Plants in the low Pi area were larger than in the high Pi area 6 weeks after planting but over the whole growth period PGCD was only slightly less in the high Pi area, resulting in little difference in overall yields between the two Pi levels.

Overall, plants grown from 0 day-degree seed tubers produced greater yields than those from 400 day-degree seed tubers, but this was not significant for individual cultivars. Six weeks after planting, 400 day-degree plants were larger than 0 day-degree plants but they senesced earlier in the autumn resulting in 400 day-degree plants having lower overall PGCD's and lower yields.

TABLE 93 Field Trial 4: Physiological age and plant dry weight on 7 July

	Day-degrees		
	0	400	S.E.D.(1 d.f.)
Plant dry weight (g)	74.2	93.7	5.72

TABLE 94 Field Trial 4: Cultivar, physiological age and plant dry weight on 7 July

	Day-degrees		
	0	400	S.E.D.(4 d.f.)
Clone 12288	61.8	88.9	
Morag	80.9	89.1	
Sante	85.6	101.3	12.78
Maris Piper	72.9	91.5	
Pentland Dell	69.9	97.5	

Mean Pf/Pi's were 1.20, 1.66, 1.72, 6.15 and 7.44 for clone 12288, Sante, Morag, Maris Piper and Pentland Dell respectively. The Pf/Pi's of clone 12288, Sante and Morag were significantly lower than those of Maris Piper and Pentland Dell as a result of their partial resistance to G. pallida. In Maris Piper and Pentland Dell plots the Pf/Pi's were significantly greater for 0 than 400 day-degree seed tubers.

4.4 GENERAL DISCUSSION OF FIELD TRIALS 1-4

4.4.1 Yield

Ware yields from the non resistant cultivars Maris Piper, Pentland Dell, Maris Bard and Estima were low in all trials, with ranges of 13-25, 3-12, 9-20 and 11-18 t/ha respectively. The partially resistant cultivars/clone Morag, Sante and 12288 had acceptable yields of 28, 33 and 36 t/ha in Field Trial 4 (Table 95).

Seed tubers of the cultivars Maris Piper, Pentland Dell, Maris Bard and Estima were purchased from Scotland and achieving early planting dates with physiologically aged seed tubers requires early delivery. The maximum temperature at which seed tubers can be safely aged is 12°C because, above this temperature, the sprout may suffer from calcium deficiency (E. J. Allen, pers. comm.), the symptoms of which are necrotic regions in the elongating region of the sprout (Dyson and Digby, 1975). At 12°C (using a basal temperature of 4°C) 7 weeks are needed after the break of dormancy to condition a seed-tuber to a physiological age of 400 day-degrees; delivery of seed stocks is therefore required at least 2 months before planting.

The earliest planting dates achieved were 21 May in 1987 and 4 May in 1988. Dyke (1956) reported that for every week that the planting of a potato crop was delayed beyond the second or third week of April there was a loss of yield of approximately 0.75

TABLE 95 Field Trials 1-4: Cultivar mean yields

		Field Trial			
	Yield	1	2	3	4
Maris Piper	Total	17.15	23.02	35.50	34.93
	Ware	13.58	19.76	26.10	25.20
Pentland Dell	Total	7.12	8.01	22.60	22.84
	Ware	2.93	3.45	12.10	10.90
Maris Bard	Total		12.63	26.70	
	Ware		9.15	20.30	
Estima	Total		15.08	24.60	
	Ware		11.47	18.00	
Clone 12288	Total				36.95
	Ware				36.44
Morag	Total				36.26
	Ware				28.53
Sante	Total				39.47
	Ware				33.05

t/ha. If this yield penalty is calculated for each field trial then the maximum yield loss was only 6.2 t/ha (Table 96). This suggests that while late planting contributed to low yields the major cause of yield loss was damage inflicted by G. pallida.

Late planting decreases yield by reducing the amount of solar radiation that can be intercepted by the crop canopy. Allen and Scott (1980) reported a close linear relationship between total and tuber dry matter yields and the amount of radiation intercepted by the crop, which is itself related to percentage ground cover (Burstall and Harris, 1983). Percentage ground cover (measured weekly from emergence to harvest) was used to calculate percentage ground cover duration, which is a crude measure of the proportion of incident solar radiation that could be intercepted by the crop canopy.

4.4.2 Physiological Age and Plant Growth

In every field trial emergence from 400 day-degree seed tubers was earlier than from 0 day-degree seed tubers. This is an effect of physiological age that has been observed by many workers (Headford, 1962; Sinha, 1974; O'Brien et al., 1983; O'Brien et al., 1986; Allen and O'Brien, 1986).

Advanced emergence lead to plants growing from physiologically aged seed tubers having larger canopies early in the growing season than plants grown from 0 day-degree seed tubers (Figs 14, 21, 26, 27, 31). The growth analysis of Maris Piper and Pentland Dell in Field Trial 1 (Table 24) revealed that from 27 to 69 days after planting (late June to mid August) plants grown from 400 day-degree seed tubers were significantly heavier than those from 0 day-degree tubers, but after mid August the differences were no longer significant. Therefore, in the early growing season, not just the canopy but the whole plant was larger from 400 than 0 day-degree seed tubers. By 50-80 days after planting there was little difference in percentage ground

TABLE 96 Field Trials 1-4: Yield loss attributable to planting beyond 14 April, according to Dyke (1956)

Field Trial	Planting Date(s)	Time after April 14		Yield loss (t/ha)
		Days	Weeks	
1	2-3 June 1987	49	7.0	5.2
2	21 May	37	5.3	4.0
	11 June 1987	58	8.3	6.2
3	3-5 May	20	2.9	2.2
	25-27 May 1988	42	6.0	4.5
4	3-5 May	20	2.9	2.2

covers between treatments and this coincided with the difference in plant dry weights between physiological ages becoming insignificant.

Peak percentage ground covers were usually greatest for 0 day-degree plants and this was especially pronounced in Maris Piper, clone 12288, Morag and Sante plots (Table 97). Where physiological age had an obvious effect on canopy development, plots grown from 400 day-degree seed tubers always senesced earlier than those from 0 day-degree seed tubers (Table 97), an effect similar to that of early planting (Allen and Scott, 1980). O'Brien et al. (1983) reported that physiologically ageing Maris Bard and Home Guard seed-tubers with up to 1200 day-degrees produced plants with smaller peak leaf areas and earlier senescence. In these trials, conducted with a maximum of 400 day-degrees ageing, it is possible that the moisture stress imposed by nematode damage to the root system is exaggerating the usual effects of physiological age on plant growth. O'Brien (1979) demonstrated that the roots of plants from physiologically old seed tubers stopped growing shortly after emergence. Therefore plants from physiologically aged seed tubers grown with the additional stress from nematode damage are especially vulnerable to shortages of water and nutrients.

4.4.3 Physiological Age and Yield

Physiological age only affected overall ware yield in Field Trials 1 and 4 (Table 98). In Field Trial 1 plants grown from 400 day-degree seed tubers outyielded 0 day-degree plots overall but not when Maris Piper and Pentland Dell were considered individually. In Field Trial 4, plants from 0 day-degree seed tubers outyielded those from 400 overall but this was not significant for any individual cultivar tested.

When total yield is considered, physiological age had significant overall effects in Field Trials 1, 3 and 4; 0 day-

TABLE 97 Field Trials 1-4: The effect of physiological age on peak percentage ground cover (PGC) and senescence

	Field Trial	Maris Piper	Pentland Dell	Maris Bard	Estima	Clone 12288	Morag	Sante
Greatest peak PGC	1	0	400					
	2	0	400	0	400			
	3	0	0	400	0			
	4	0	0			0	0	0
Earliest senescence	1	-	-					
	2	-	-	400	-			
	3	400	400	400	400			
	4	400	400			400	400	400

0 = 0 day-degree plots
400 = 400 day-degree plots
- = no obvious difference between 0 and 400 day-degree plots

TABLE 98 Field Trials 1-4: Physiological age and ware yield, total yield and PGCD

	Field Trial	Overall	Maris Piper	Pentland Dell	Maris Bard	Estima	Clone 12288	Morag	Sante
Greatest ware yield	1	400	NS	NS					
	2	NS	NS	NS	NS	NS			
	3	NS	NS	NS	NS	NS			
	4	0	NS	NS			NS	NS	NS
Greatest PGCD	1	400	NS	400					
	2	NS	NS	NS	NS	NS			
	3	NS	NS	NS	NS	NS			
	4	0	NS	NS			NS	NS	NS
Greatest total yield	1	400	400	400					
	2	NS	NS	NS	0	NS			
	3	0	NS	NS	NS	NS			
	4	0	NS	NS			NS	NS	NS

NS = difference not significant

degree seed tubers outyielding 400 in Field Trials 3 and 4 and 400 day-degree seed tubers outyielding 0 in Field Trial 1.

Observing individual cultivars over all four field trials (Table 98) physiological age had no significant effect on ware yield in any case. Therefore physiological age has little effect on yield.

Bean and Allen (1980) suggested that the effects of physiological age on growth and yield could be explained by the variation in the amount of light intercepted during the growth period. In Field Trials 1 and 4, where physiological age affected overall ware and total yields, there was a similar effect on PGCD (Table 98) which would support this view.

4.4.4 Tolerance

The degree of tolerance of a cultivar to attack by PCN may be expressed as the yield loss of a test cultivar relative to a standard cultivar under the same conditions of nematode challenge (Evans and Haydock, 1990), and Maris Piper is a suitable (tolerant) reference cultivar.

In Field Trial 1 there was no significant difference in Pi between cultivars; in Field Trial 2 Pentland Dell plots had a higher Pi than those of Estima, Maris Piper and Maris Bard, although the latter three cultivars were not significantly different from one another; in Field Trials 3 and 4, within each of the low and high Pi areas, Pi's were not significantly different between cultivars. Therefore, if we accept that in Field Trial 2 Pentland Dell will appear less tolerant than it actually is due to the greater nematode challenge, then the ware yield of cultivars can be expressed as a percentage of Maris Piper and tolerance levels compared (Table 99).

This table suggests that tolerance decreases in the order-clone 12288, Sante, Morag, Maris Bard, Pentland Dell. Clone 12288, Sante and Morag were more tolerant than Maris Piper while

TABLE 99 Field Trials 1-4: Ware yields as a percentage of the tolerant cultivar Maris Piper

		Field Trial					
		1	2	3		4	
				L	H	L	H
		Ware yield (t/ha)					
Maris Piper	Overall	13.6	19.8	32.1	20.0	23.6	26.8
	0	10.5	18.0	32.4	20.4	26.7	28.1
	400	14.7	21.5	31.9	22.9	20.5	25.5
		Yield as a percentage of Maris Piper					
Pentland Dell	Overall	22	17	64	18	41	45
	0	11	17	71	11	50	39
	400	34	18	57	25	30	52
Maris Bard	Overall		46	91	56		
	0		59	90	54		
	400		35	91	59		
Estima	Overall		58	85	42		
	0		69	93	45		
	400		49	78	40		
Clone 12288	Overall					151	139
	0					157	143
	400					142	135
Morag	Overall					123	105
	0					127	117
	400					118	90
Sante	Overall					143	120
	0					127	129
	400					165	111

L = low Pi, H = high Pi

Maris Bard, Estima and Pentland Dell were less tolerant. There was little difference in the tolerance status of Maris Bard and Estima. The effect of physiological age on the tolerance of a cultivar is variable (Table 99): physiological age had little effect on the tolerance of Pentland Dell in Field Trial 2; in Field Trial 1, however, and the high Pi areas of Trials 3 and 4, tolerance was increased with 400 day-degree seed tubers compared to 0 day-degree seed tubers, but in the low Pi areas of Trials 3 and 4 tolerance was decreased. For Maris Bard physiological age had little effect on tolerance in the low Pi area of Trial 3; in the high Pi area 400 day-degree plants appeared more tolerant than 0 and in Trial 2 treatment with 400 day-degrees decreased plant tolerance. The tolerance of Estima was decreased by physiologically ageing seed tubers to 400 day-degrees in both Trials 2 and 3. In both the high and low Pi areas 400 day-degree treatments were less tolerant than 0 in clone 12288 and Morag plots. For Sante 400 day-degree plants were more tolerant in the low Pi area but less tolerant in the high Pi area than plants grown from 0 day-degree seed tubers.

Evans and Trudgill (1978) gave a practical definition of tolerance of nematode attack as tolerant cultivars being those which suffer a smaller reduction in yield at high nematode densities. Field Trials 3 and 4 both contained low (69 and 115 eggs per g of soil respectively) and high (280 and 309 eggs per g of soil respectively) Pi treatments in which there were no significant differences in Pi within each level but the high Pi was significantly greater than the low Pi. Therefore the tolerance of a cultivar can be assessed by expressing the yield in the high Pi area as a percentage of that in the low Pi area (Table 100). In Field Trial 3 Maris Piper was the most tolerant cultivar followed by Maris Bard, Estima and Pentland Dell, which is the same as the ratings from Table 99. However, in Field Trial

TABLE 100 Field Trials 3 and 4: Ware yield in the high Pi area as a percentage of ware yield in the low Pi area

		Field Trial 3		Field Trial 4	
	Day-degrees	low Pi (t/ha)	high Pi (%)	low Pi (t/ha)	high Pi (%)
	Overall	32.1	62	23.6	114
Maris Piper	0	32.4	63	26.7	105
	400	31.9	62	20.5	125
	Overall	20.6	17	9.7	125
Pentland Dell	0	22.9	10	13.3	83
	400	18.3	27	6.1	216
	Overall	29.2	39		
Maris Bard	0	29.3	38		
	400	29.1	40		
	Overall	27.4	31		
Estima	0	30.1	30		
	400	24.8	32		
	Overall			35.6	105
Clone 12288	0			42.0	96
	400			29.2	118
	Overall			29.0	97
Morag	0			33.9	97
	400			24.2	95
	Overall			33.8	96
Sante	0			33.9	107
	400			33.7	84

4, Pentland Dell was the most tolerant followed by Maris Piper, clone 12288 and Sante. When physiological age is considered (Table 71) the reason for the unexpectedly high tolerance rating of Pentland Dell can be seen. Zero day-degree plots had ware yields of 13.3 and 11.0 t/ha in the low and high Pi areas but in the 400 day-degree plots the high Pi area (280 eggs per g of soil) had a ware yield of 13.2 t/ha, which was, unexpectedly, over twice that from the low Pi area (69 eggs per g soil, 6.0 t/ha).

In Field Trial 3 (Table 100) physiological age had little effect on the tolerance of Maris Piper, Maris Bard and Estima. However, physiologically ageing seed tubers to 400 day-degrees increased the tolerance of Pentland Dell in both Field Trials 3 and 4. In Field Trial 4 the tolerance of Maris Piper and clone 12288 were increased by physiological ageing; physiological age had little effect on the tolerance of Morag and decreased that of Sante.

4.4.5 Nematode Multiplication

In Field Trial 1 there were no significant differences in Pi, Pf and Pf/Pi between cultivars, physiological ages and aldicarb treatment. Pentland Dell plots in Trial 2 had a significantly higher Pi than the other cultivars and as a result of the density dependent nature of nematode multiplication (Seinhorst, 1966; Ferris, 1985; LaMondia and Brodie, 1986) this cultivar had the lowest Pf/Pi ratio. There were no overall differences in Pi, Pf and Pf/Pi between physiological ages, aldicarb treatments and planting dates in Field Trial 2.

The low and high Pi areas of both Field Trials 3 and 4 had significantly different Pi's but were uniform in nematode population density within Pi levels. Pf/Pi ratios were larger in the low than the high Pi areas. In Trial 3 there were no significant differences in Pf/Pi ratios between cultivar,

physiological age or planting date treatments.

Field Trial 4 contained partially resistant as well as non resistant cultivars and in common with Field Trials 1-3 Pf/Pi generally decreased with increasing Pi as has been found by other workers with G. pallida infested soil (Phillips, 1984; Seinhorst, 1984; Phillips et al., 1988) due to increasing intraspecific competition and root damage at higher Pi's. Physiological age had no significant effect on Pf/Pi for clone 12288, Morag or Sante but in Maris Piper and Pentland Dell plots (Table 83) Pf/Pi was significantly lower in 400 than 0 day-degree plots. The partially resistant cultivars/clone Morag, Sante and clone 12288 had significantly lower Pf/Pi's than the non resistant cultivars Maris Piper and Pentland Dell (Table 101).

TABLE 101 Field Trials 1-4: Cultivar, Pi and Pf/Pi

Field Trial		Maris Piper	Pentland Dell	Maris Bard	Estima	Clone 12288	Morag	Sante
1	Pi	350	333					
	Pf/Pi	1.92	2.03					
2	Pi	310	420	327	330			
	Pf/Pi	2.97	1.54	1.71	3.10			
3	Pi	163	187	167	181			
	Pf/Pi	19.0	11.7	10.2	12.3			
4	Pi	225	212			213	200	209
	Pf/Pi	6.15	7.44			1.20	1.72	1.66

CHAPTER 5

THE EFFECT OF TEMPERATURE ON THE RATE OF DEVELOPMENT OF *G. PALLIDA* AND *G. ROSTOCHIENSIS* FROM JUVENILE (J2) INOCULA

CHAPTER 5 THE EFFECT OF TEMPERATURE ON THE RATE OF DEVELOPMENT OF G. PALLIDA AND G. ROSTOCHIENSIS FROM JUVENILE (J2) INOCULA

5.1 MATERIALS AND METHODS

Experimental Design

This experiment was conducted in six thermostatically controlled water-baths, in a glasshouse at Rothamsted, maintained at 6, 8, 10, 13, 16 and 19°C by the circulation of cooled or heated water. Individual water-baths were divided into three areas, representing experimental blocks, each containing one randomly assigned replicate of the treatments (sampling dates). Water-baths at 6, 8, 10 and 13C contained 60 plants (2 nematode species X 3 replicates X 10 sampling intervals) while those at 16 and 19°C contained 66 plants (2 species X 3 replicates X 11 sampling intervals - Fig. 32).

Potato Plants

Desiree tubers were physiologically aged at 10°C in an illuminated potato store until they had grown sprouts of 5-10mm length. Single sprouts attached to a plug of tuber were cut using a scalpel and planted singly in a plastic cup of 0.3l capacity containing moist sand. They were transferred to 16°C water baths to grow for 16 days prior to inoculation with nematodes.

Nematode Inocula

Cysts of G. pallida and G. rostochiensis were soaked for 7 days in distilled water which was replaced by a 1:3 dilution of potato root diffusate and distilled water for a further 7 days. Hatched juveniles (JJ2) were removed in suspension and kept at 3°C until required for inoculation.

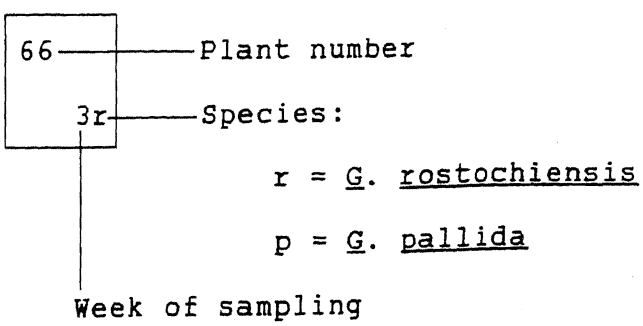
Inoculation

Juveniles (JJ2) were inoculated into the sand surrounding the growing plants on 24 April using a wide bore hypodermic

FIG. 32 Experimental design in a water bath

Example = 19℃

1 4p	2 2.5p	3 5p	4 1p	5 5r	6 4r	7 5.5p	
8 5.5r	9 4.5r	10 3.5r	11 2r	12 3p	13 4.5r	14 1r	Block 1
15 1.5r	16 6r	17 1.5r	18 2p	19 3r	20 3.5r	21 6p	
22 2.5r	23 4.5r	24 4.5r	25 2.5p	26 6p	27 4p	28 3r	
29 5r	30 5.5p	31 1.5p	32 5p	33 1p	34 2.5r	35 2p	Block 2
36 5.5r	37 3.5p	38 6r	39 3p	40 3.5r	41 1r	42 4r	
43 1.5r	44 2r	45 3.5r	46 2.5r	47 4.5r	48 1.5r	49 5r	
50 4.5r	51 2r	52 5.5p	53 1p	54 2.5p	55 5p	56 3p	Block 3
57 4p	58 2p	59 1r	60 6p	61 4r	62 5.5r	63 6r	
64 3.5p	65 1.5p	66 3r					



needle attached to a 2ml syringe. Four inoculations were made, each containing approximately 300 juveniles suspended in 1ml of distilled water. The water-baths were left at 16°C for 24 hours after inoculation, to allow juveniles to invade the roots, before the baths were adjusted to their operating temperatures.

Water and Fertiliser

As the plastic pots used were watertight, care had to be taken to prevent the sand from being waterlogged; this was achieved by frequent applications of small volumes of water. From 25 April all plants received a solution of a balanced fertiliser containing nitrogen, phosphate and potash (10:10:27; Phostrogen) along with Mg, Fe and Mn, at 4g/9 litres of tap water. Air temperature in the glasshouse was within the range 10-31°C during the experiment.

Sampling

The three replicates of plants inoculated with the two nematode species and kept at 6, 8, 10 and 13°C were destructively sampled at weekly intervals for 10 weeks after inoculation; those at 16 and 19°C were sampled twice weekly for 5.5 weeks. Plants were removed from the plastic cups and the sand gently washed from the roots. Males were extracted from the sand and the wash water by elutriation in a fluidising column (Trudgill, Evans and Faulkner, 1973) for 5 minutes at 2l/minute followed by 10 seconds at 5l/minute. Males were collected on a bank of sieves of aperture ranging from 150 to 53µm.

Processing

Plants were dissected and their roots, shoots and stolons weighed. The root system was cut into 1cm lengths, thoroughly mixed and a 2g sub sample preserved in FAA. Nematodes in the root sample were stained by boiling for 3 minutes in 0.05% acid fuchsin in equal volumes of glycerol, lactic acid and distilled water (Bridge, Page and Jordan, 1982). Excess lactoglycerol stain

was removed by washing in hot water prior to macerating the root sample for 30 seconds in an "Atomix" blender. Sample volume was made up to 200ml with distilled water, agitated, and a 20ml sub sample taken in which nematodes were counted and their development stage visually assessed.

5.2 RESULTS

The mean number of nematodes per root system and their developmental stages at each temperature and sampling date are shown in Tables 102 to 107. A key to abbreviations used in these tables is given on page 184.

5.3 DATA ANALYSIS

Data from Tables 102 to 107 has been analysed by two different methods to estimate the basal temperature for development of *G. pallida* and *G. rostochiensis*. All data was analysed using Genstat 5, Release 2.1.

5.3.1 Method 1 - Probability and Regression Analysis

Chi-squared analysis was used to predict the probability of nematodes developing to the next developmental stage for each water bath temperature. Probability data were produced for J2 to J3, J3 to J4 and J4 to J5 at 6, 8, 10, 13, 16 and 19°C (Table 108) and these are illustrated graphically in Figure 33. For both species, as temperature increases the probability of developing to the next stage is increased. At the lower temperatures (6 and 8°C) *G. pallida* is more likely to develop than *G. rostochiensis*, suggesting that the former species is better adapted to low temperatures.

However, even at the lowest temperature of 6°C, both species were still developing and therefore a linear regression was used to estimate the basal temperature for development of both species i.e. where the probability of a nematode developing to the next stage was zero. Table 109 gives the results of these regressions

Key for Tables 102 to 107

W = sampling week

S = species : R = *G. rostochiensis*

P = *G. pallida*

Numbers of nematodes per root system:-

J2 = second stage juvenile

J3 = third stage juvenile

J4M = fourth stage male juvenile

J4F = fourth stage female juvenile

J4M+J4F = total number of fourth stage male and female juveniles

J5M = adult male

J5F = adult female

T = total number of all developmental stages

TABLE 102 PCN development at 6℃ (mean number of nematodes per root system)

W	S	Developmental stage									Plant fresh weight	
		J2	J3	J4 M	J4 F	J4M +J4F	J5 M	J5 F	J5M +J5F	T	Mean root wt(g)	Total plant wt(g)
1	R	77	0	0	0	0	0	0	0	77	2.84	4.80
	P	93	0	0	0	0	0	0	0	93	2.63	4.49
2	R	59	23	0	0	0	0	0	0	82	2.01	3.83
	P	85	3	0	0	0	0	0	0	88	2.07	4.03
3	R	48	0	0	0	0	0	0	0	48	2.07	4.17
	P	97	47	0	0	0	0	0	0	143	1.59	3.24
4	R	93	10	0	3	3	3	3	7	113	1.78	4.02
	P	80	160	0	0	0	3	0	3	243	1.59	3.53
5	R	33	7	28	8	36	0	0	0	76	1.86	3.85
	P	88	61	7	0	7	0	0	0	156	1.81	3.43
6	R	30	37	0	0	0	0	0	0	67	1.79	4.35
	P	10	58	60	26	86	74	8	82	236	1.91	4.42
7	R	44	55	3	3	7	0	0	0	106	1.93	4.15
	P	23	47	90	43	133	63	0	63	267	1.91	4.29
8	R	15	11	15	39	54	26	0	26	106	3.26	5.32
	P	27	43	37	67	103	150	0	150	323	1.78	4.45
9	R	27	47	10	3	13	3	0	3	90	1.67	4.44
	P	13	23	23	47	70	233	0	233	340	1.71	4.38
10	R	10	17	17	17	34	23	0	23	84	1.91	5.11
	P	7	3	20	67	87	207	0	207	303	1.79	5.22
SED (9df)		27.6	22.5			24.0			18.2	53.7	0.340	0.711

TABLE 103 PCN development at 8°C (mean number of nematodes per root system)

W	S	Developmental stage									Plant fresh weight	
		J2	J3	J4 M	J4 F	J4M +J4F	J5 M	J5 F	J5M +J5F	T	Mean root wt(g)	Total plant wt(g)
1	R	47	0	0	0	0	0	0	0	47	2.02	3.83
	P	97	0	0	0	0	0	0	0	97	1.96	3.58
2	R	119	3	4	0	4	0	0	0	127	2.30	4.72
	P	174	22	0	0	0	0	0	0	197	2.06	4.30
3	R	74	49	0	0	0	0	0	0	123	2.31	5.19
	P	150	116	0	0	0	0	0	0	265	1.99	4.16
4	R	80	93	0	0	0	0	0	0	173	1.83	3.73
	P	50	193	27	0	27	3	0	3	273	1.53	3.69
5	R	63	97	0	3	3	3	0	3	167	2.17	4.75
	P	30	90	154	40	194	13	0	13	328	1.67	3.90
6	R	7	79	33	18	51	21	0	21	159	2.39	5.95
	P	13	14	27	82	109	223	0	223	359	2.10	5.00
7	R	26	20	14	19	33	7	0	7	85	2.12	5.24
	P	4	17	28	95	123	183	0	183	328	1.82	4.09
8	R	10	3	30	57	87	70	0	70	170	1.85	3.98
	P	17	13	17	47	63	227	3	230	323	1.63	3.79
9	R	8	38	20	46	66	105	8	114	226	2.09	4.51
	P	20	10	20	30	51	189	27	216	297	1.91	4.63
10	R	14	3	14	10	24	31	7	39	80	2.01	5.48
	P	17	3	0	27	27	70	37	107	153	1.78	5.53
SED (9df)		29.2	30.5			24.7			22.9	54.8	0.339	0.795

TABLE 104 PCN development at 10°C (mean number of nematodes per root system)

W	S	Developmental stage J2	J3	J4 M	J4 F	J4M +J4F	J5 M	J5 F	J5M +J5F	T	Plant fresh weight Mean root wt(g)	Total plant wt(g)
1	R	54	0	0	0	0	0	0	0	54	2.14	4.15
	P	118	0	0	0	0	0	0	0	118	2.07	3.78
2	R	99	20	0	0	0	0	0	0	119	2.27	4.74
	P	260	23	0	0	0	0	0	0	283	1.50	3.50
3	R	21	72	49	8	57	7	0	7	157	2.15	4.19
	P	12	39	96	65	161	257	5	262	473	2.49	5.62
4	R	14	42	108	14	121	38	0	38	216	1.87	3.97
	P	7	70	117	113	230	120	0	120	427	1.73	3.74
5	R	13	13	30	40	70	70	0	70	167	1.52	3.11
	P	18	14	42	54	95	216	3	219	346	1.85	5.02
6	R	3	3	17	7	23	93	7	100	130	1.44	3.81
	P	24	7	10	20	30	100	23	123	184	1.74	4.10
7	R	17	3	3	23	27	70	0	70	117	1.66	4.76
	P	7	7	7	13	20	37	17	50	83	1.59	4.59
8	R	0	7	3	10	13	23	17	40	60	1.75	5.17
	P	0	0	3	3	7	20	13	33	40	1.53	4.93
9	R	0	0	0	10	10	14	10	24	35	2.00	6.24
	P	3	0	0	7	7	27	23	50	60	1.84	5.69
10	R	4	7	0	11	11	4	29	33	55	2.05	7.27
	P	20	0	0	13	13	13	13	27	60	1.78	5.44
SED (9df)		23.9	16.2			24.2			46.6	57.1	0.296	0.922

TABLE 105 PCN development at 13°C (mean number of nematodes per root system)

W	S	Developmental stage									Plant fresh weight	
		J2	J3	J4 M	J4 F	J4M +J4F	J5 M	J5 F	J5M +J5F	T	Mean root wt(g)	Total plant wt(g)
1	R	78	0	0	0	0	0	0	0	78	2.51	4.37
	P	223	20	0	0	0	0	0	0	248	7.70	5.30
2	R	87	81	0	0	0	0	0	0	168	2.82	5.88
	P	77	156	0	0	0	0	0	0	232	2.63	5.24
3	R	26	28	77	48	125	139	0	139	318	2.44	5.31
	P	11	13	96	61	157	331	0	331	512	2.14	4.75
4	R	3	14	37	87	124	67	21	88	229	1.93	5.51
	P	14	60	21	106	127	125	19	144	346	2.12	5.51
5	R	10	20	20	44	64	44	3	48	143	1.82	4.90
	P	56	25	17	147	164	121	17	138	383	2.10	6.46
6	R	11	12	19	78	97	19	11	31	150	2.08	7.10
	P	15	26	23	98	121	66	18	84	245	2.03	7.48
7	R	22	10	0	72	72	56	0	56	161	2.45	9.36
	P	17	28	14	92	105	93	3	97	247	1.85	6.78
8	R	0	3	0	120	120	40	0	40	163	1.79	7.78
	P	10	3	10	80	90	110	0	0	213	1.69	6.85
9	R	7	0	4	47	51	24	0	24	79	1.95	8.60
	P	18	17	21	53	74	53	7	60	169	1.99	8.08
10	R	7	10	3	60	64	30	0	30	111	1.86	10.58
	P	20	10	0	37	37	40	3	43	110	1.81	11.05
SED (9df)		24.5	25.7			41.1			46.7	76.6	0.297	1.073

TABLE 106 PCN development at 16°C (mean number of nematodes per root system)

W	S	Developmental stage									Plant fresh weight	
		J2	J3	J4 M	J4 F	J4M +J4F	J5 M	J5 F	J5M +J5F	T	Mean root wt(g)	Total plant wt(g)
1	R	79	8	0	0	0	0	0	0	87	2.27	4.78
	P	144	118	0	0	0	0	0	0	262	2.40	4.65
1.5	R	44	77	7	5	11	14	0	14	146	2.40	4.41
	P	43	89	26	7	33	3	0	3	168	2.36	5.11
2	R	15	48	230	16	246	50	0	50	360	2.23	4.49
	P	42	128	204	9	213	117	0	117	501	2.52	4.67
2.5	R	11	57	54	53	107	232	0	232	407	2.16	5.08
	P	0	14	24	162	186	361	0	361	561	2.57	5.85
3	R	13	25	26	59	85	190	0	190	313	2.38	5.33
	P	3	31	14	114	128	316	7	323	485	2.56	5.98
3.5	R	8	9	20	9	29	126	51	177	223	2.26	4.83
	P	27	23	29	43	72	196	30	227	349	2.54	6.10
4	R	10	3	19	18	37	65	46	110	161	1.98	5.13
	P	7	0	0	16	16	99	70	170	193	2.60	5.94
4.5	R	6	3	3	3	7	27	26	53	69	2.44	6.50
	P	17	7	14	14	28	44	25	69	122	2.17	6.01
5	R	3	7	4	4	8	14	15	29	47	1.98	5.19
	P	10	18	0	0	0	9	8	17	44	2.73	8.99
5.5	R	13	0	0	8	8	14	3	18	38	2.05	5.67
	P	11	11	0	0	0	12	8	20	43	2.38	6.69
6	R	3	7	0	3	3	14	3	18	32	2.05	7.18
	P	7	3	0	3	3	14	7	20	34	1.91	6.33
SED (10df)		15.6	24.1			33.5			61.5	73.3	0.400	1.084

TABLE 107 PCN development at 19℃ (mean number of nematodes per root system)

W	S	Developmental stage									Plant fresh weight	
		J2	J3	J4 M	J4 F	J4M +J4F	J5 M	J5 F	J5M +J5F	T	Mean root wt(g)	Total plant wt(g)
1	R	123	149	0	0	0	0	0	0	273	2.90	5.63
	P	294	170	4	0	4	0	0	0	468	2.25	4.30
1.5	R	15	5	30	8	38	15	0	15	72	2.22	4.55
	P	22	32	69	19	88	32	0	32	174	2.12	4.39
2	R	13	43	30	177	207	220	3	223	487	1.68	4.08
	P	0	32	110	119	230	192	0	192	454	2.50	5.17
2.5	R	7	3	13	109	122	250	71	320	453	1.93	4.20
	P	9	4	21	68	90	263	9	272	375	2.67	5.78
3	R	7	0	0	25	25	49	40	89	121	2.09	5.43
	P	0	4	8	16	23	190	80	270	297	2.77	6.07
3.5	R	3	0	7	21	27	19	60	79	110	2.55	6.15
	P	4	8	4	28	32	62	109	172	215	2.44	6.09
4	R	0	0	0	4	4	13	46	59	63	2.56	6.24
	P	0	3	18	7	26	14	60	74	103	2.08	5.21
4.5	R	4	0	0	15	15	8	4	12	31	2.29	6.80
	P	0	0	0	11	11	11	21	32	43	2.17	5.76
5	R	12	4	0	0	0	11	0	11	27	2.29	6.80
	P	10	0	7	7	14	18	7	25	49	2.05	6.23
5.5	R	4	9	4	16	21	13	17	31	64	2.44	7.44
	P	0	0	3	3	7	0	8	8	15	2.48	8.61
6	R	3	3	0	3	3	13	7	20	30	1.57	6.48
	P	0	10	3	3	7	13	13	27	43	1.53	7.62
SED (10df)		16.1	18.1			27.8			33.4	61.4	0.322	0.881

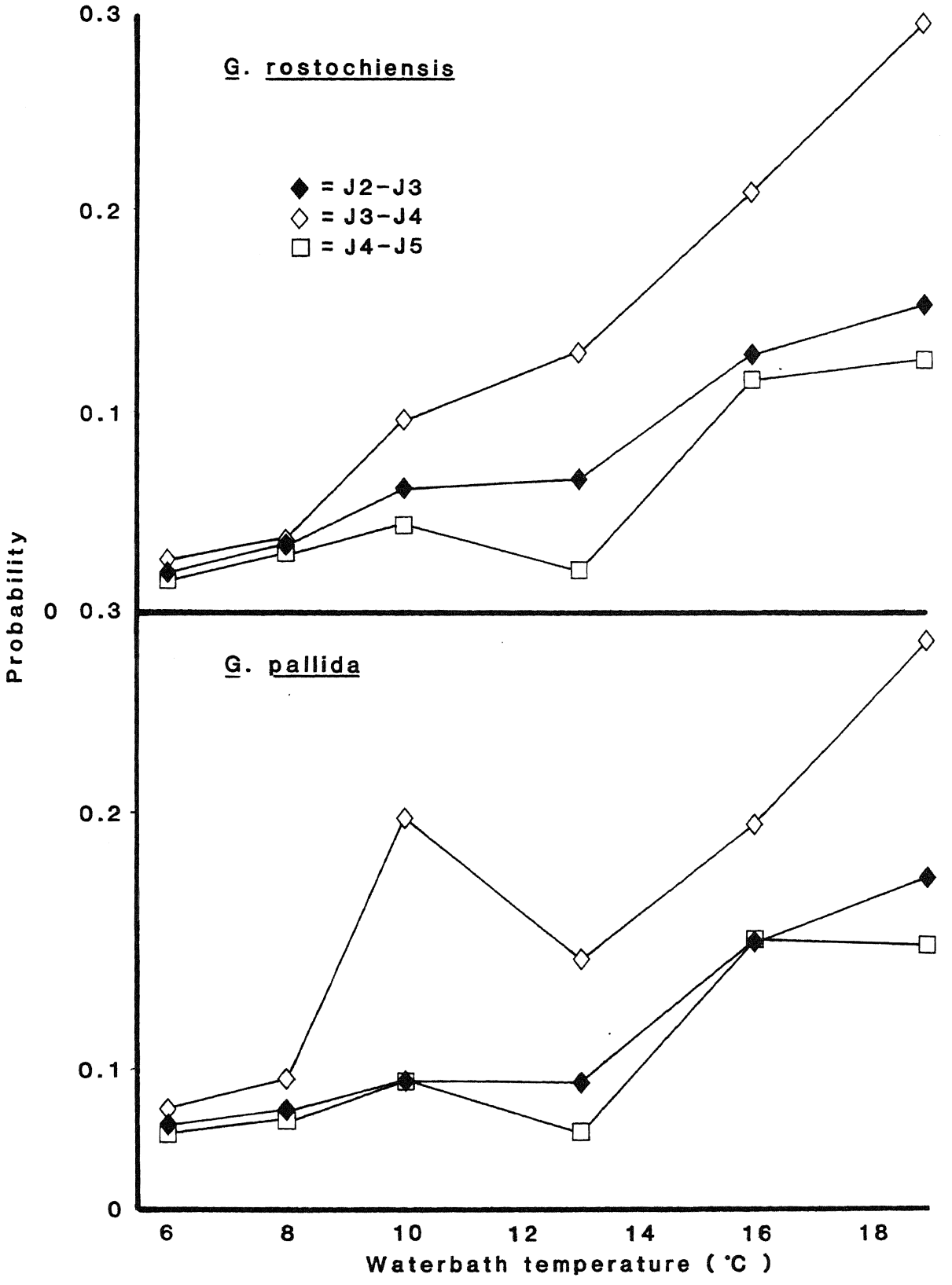
TABLE 108 Water bath temperature and the probability of nematodes maturing to the next developmental stage

Species	Developmental stage transition	P or SE	Water bath temperature (°C) 6	8	10	13	16	19
P	J2 - J3	P	0.0415	0.0512	0.0643	0.0636	0.1354	0.1632
		SE	0.0023	0.0029	0.0045	0.0046	0.0094	0.0097
	J3 - J4	P	0.0512	0.0670	0.1961	0.1258	0.1941	0.2858
		SE	0.0038	0.0054	0.0294	0.0167	0.0203	0.2801
	J4 - J5	P	0.0394	0.0467	0.0651	0.0409	0.1364	0.1342
		SE	0.0035	0.0042	0.0069	0.0043	0.0139	0.0111
R	J2 - J3	P	0.0197	0.0348	0.0627	0.0675	0.1302	0.1565
		SE	0.0023	0.0028	0.0059	0.0043	0.0092	0.0093
	J3 - J4	P	0.0272	0.0356	0.0977	0.1382	0.2113	0.2971
		SE	0.0051	0.0041	0.0138	0.0162	0.0234	0.0299
	J4 - J5	P	0.0170	0.0306	0.0449	0.0220	0.1176	0.1279
		SE	0.0056	0.0050	0.0065	0.0021	0.1197	0.0109

P = G. pallida

R = G. rostochiensis

Fig. 33 Waterbath temperature and the probability of nematodes maturing to the next developmental stage



and estimates the basal temperature for development for each species. The mean values are 2.5 and 5.1°C for G. pallida and G. rostochiensis respectively.

5.3.2 Method 2 - Least Variance Analysis

As the rate of development of a nematode is determined by accumulated heat units rather than temperature per se (Jones and Parrott, 1969), the number of heat units required to mature from one developmental stage to the next should be independent of ambient temperature. For example, at 10°C the transition from J4 to J5 will take fewer days than at 5°C although the number of day-degrees will be similar at both temperatures.

The least variance analysis assumed a basal temperature for development (1.0 to 5.0°C in 0.1°C intervals) and calculated the number of day-degrees above this temperature that had accumulated from the peak number of nematodes found at one developmental stage to the peak number at the next stage. This was calculated for all developmental stage transitions at each temperature for both PCN species. The best estimate of the basal temperature for development was when the variance between the number of day-degrees required for any given developmental stage was least between temperature treatments. Data are shown in Tables 110 and 111 for G. pallida and G. rostochiensis respectively. Least variance occurs at 2.7 - 4.4°C (mean 3.5°C) for G. pallida and at 3.4 - 4.7°C (mean 4.1°C) for G. rostochiensis. Using the mean basal temperatures (of all developmental stages) of 3.5 and 4.1°C for G. pallida and G. rostochiensis respectively, the number of day-degrees required to peak occurrence of each developmental stage was calculated for all temperature treatments (Tables 112 and 113). Table 114 is a summary of Tables 110 to 113.

5.4 DISCUSSION

The major limitation of this experiment was that a water

TABLE 109 Regression analysis of probability data from Table 108 with Y-Zero representing the estimated basal temperature for development

Species	Developmental stage transition		Regression coefficient	S.E.	% Variance accounted for	Y-Zero
P	J2 - J3	Constant	-0.0281	0.0220		
		Temp.	0.0095	0.0017	85.7	2.938
	J3 - J4	Constant	-0.0370	0.0550		
		Temp.	0.0159	0.0043	71.7	2.333
	J4 - J5	Constant	-0.0185	0.0308		
		Temp.	0.0080	0.0024	66.6	2.324
					Mean	2.532
R	J2 - J3	Constant	-0.0494	0.0145		
		Temp.	0.0107	0.0011	94.6	4.630
	J3 - J4	Constant	-0.1175	0.0201		
		Temp.	0.0210	0.0016	97.3	5.593
	J4 - J5	Constant	-0.0456	0.0310		
		Temp.	0.0088	0.0024	70.9	5.179
					Mean	5.134

P = G. pallida

R = G. rostochiensis

TABLE 110 Estimated basal temperatures of development for G. pallida with associated day-degrees at each water bath temperature

Developmental stage		J2	J3	J4	J5	Mean
Temperature of minimum variance (°C)		2.7	4.4	3.4	3.6	3.5
		Day-degrees above temperature of minimum variance				
Water bath temperature (°C)	6	63	43	125	149	
	8	64	97	156	242	
	10	88	151	178	122	
	13	52	103	326	179	
	16	67	139	151	198	
	19	82	73	187	246	
	S.D.	13.3	40.3	71.6	49.9	

TABLE 111 Estimated basal temperatures of development for G. rostochiensis with associated day-degrees at each water bath temperature

Developmental stage		J2	J3	J4	J5	Mean
Temperature of minimum variance	(°C)	3.4	4.4	4.7	3.8	4.1
		Day-degrees above temperature of minimum variance				
Water bath temperature (°C)	6	70	77	44	121	
	8	55	122	181	260	
	10	79	106	143	254	
	13	115	103	158	175	
	16	63	104	136	195	
	19	78	73	172	243	
	S.D.	20.9	19.0	49.0	55.0	

TABLE 112 The number of accumulated day-degrees above the mean basal temperature for development of 3.5℃ for G. pallida from JJ2 inocula to peak numbers of JJ2, JJ3, JJ4 and JJ5 in potato roots

Water bath temperature (℃)	Developmental stage J2	J3	J4	J5
6	48	68	120	155
8	54	122	153	248
10	78	176	176	124
13	48	114	323	181
16	63	150	150	200
19	78	78	186	248
S.D.	14.0	41.3	71.6	50.0
Mean	62	118	185	193

TABLE 113 The number of accumulated day-degrees above the mean basal temperature for development of 4.1°C for G. rostochiensis from JJ2 inocula to peak numbers of JJ2, JJ3, JJ4 and JJ5 in potato roots

		Developmental stage			
		J2	J3	J4	J5
Water bath temperature (°C)	6	51	91	65	104
	8	47	133	214	242
	10	71	112	159	242
	13	107	107	169	169
	16	60	107	143	190
	19	75	75	179	238
	S.D.	21.7	20.0	50.0	55.0
	Mean	68	104	155	197

TABLE 114 The mean number of accumulated day-degrees above the mean basal temperature for development required from J2 inocula to peak numbers of JJ2, JJ3, JJ4 and JJ5 in potato roots

Species		G. pallida	G. rostochiensis
Basal temperature for development		3.5	4.1
Developmental stage	J2	62	68
	J3	118	104
	J4	185	155
	J5	193	197

bath temperature sufficiently low to prevent nematode development could not be achieved. However, estimates of the basal temperatures for development for G. pallida and G. rostochiensis were obtained, being 2.5 and 5.1°C by method 1 and 3.5 and 4.1°C by the second method. Both methods give a lower basal temperature for G. pallida than for G. rostochiensis, suggesting that the former is better adapted to lower temperatures and colder environments. This is in agreement with the findings of Parrott and Berry (1976), Berry et al. (1978), Foot (1978) and Mugniery (1978).

Many workers have used "assumed" basal temperatures for development e.g. 4.4°C by Jones and Parrott (1969) and Tiilikkala (1987); 6°C by Hansen and Jakobsen (1985) and Magnusson (1986); 10°C by Greco et al. (1988), Philis (1980) and Webley and Jones (1981). However, Mugniery (1978) found basal temperatures for the development of G. pallida and G. rostochiensis to be 3.9 and 6.2°C respectively. Langeslag et al. (1982) observed basal temperatures of 4.5, 5.3 and 6.8°C for three populations of G. pallida and 5.9 and 6.3°C for two populations of G. rostochiensis. Estimates from this experiment agree well with those of Mugniery (1978) and Langeslag et al. (1982), suggesting that the basal temperature of 10°C used by Webley and Jones (1981) is too high for the U.K.

The estimated numbers of day-degrees needed to reach peak numbers of each stage of the life cycle (Table 114) are very similar for the two species, with both G. pallida and G. rostochiensis requiring approximately 200 day-degrees above 3.5 and 4.1°C respectively from hatched JJ2 in the soil to peak numbers of JJ5 found in the roots. Webley and Jones (1981) reported that females of G. pallida required fewer day-degrees to develop than those of G. rostochiensis. However, they used the same basal temperature (10°C) for both species and, as the basal temperature for G. pallida was probably lower than for G.

rostochiensis, this would have effectively underestimated the number of day-degrees required for the development of G. pallida relative to G. rostochiensis. The day-degree figures estimated in this experiment do not represent a full generation, so the total development time is an underestimate of the true figure needed for accurate phenological control of PCN as suggested by Webley and Jones (1981) or to predict the potential effect of increased soil temperatures (e.g. as a result of the "greenhouse effect") on PCN life cycles.

CHAPTER 6

THE RELEASE AND QUANTIFICATION OF ANTIGEN FROM *G. PALLIDA* CYSTS

CHAPTER 6 THE RELEASE AND QUANTIFICATION OF ANTIGEN FROM G. PALLIDA CYSTS

6.1 INTRODUCTION AND OBJECTIVES

In common with other immunological studies of plant parasitic nematodes, both the polyclonal antiserum (rabbit, PC 266) and monoclonal antibodies (rat, MR8/4.1) used in this study were raised to G. pallida by the inoculation of whole body homogenates (M. P. Robinson, pers. comm.). Their specificity is unknown although it is possible that they bind to epitopes on the juvenile's (J2) cuticle. Before juvenile (J2) antigen can be detected the tough cyst wall must be removed and the JJ2 liberated from their egg shells.

The cyst wall consists mainly of collagen-like proteins (72% calculated from the nitrogen content) with small amounts of glucosamine, lipid, carbohydrate and inorganic matter (Clarke, 1968). This is very similar in chemical content to the egg-shell (Clarke, Cox and Shepherd, 1967).

The objectives of this preliminary study are:-

1) To determine the optimum concentrations of reagents for use in an ELISA (either competitive or indirect) to detect G. pallida antigen.

2) To investigate the possibility of disrupting cyst walls/egg shells by an easy to use, repeatable, cheap chemical method.

3) To determine if ELISA can be used to detect G. pallida antigen released by the chemical disruption of the cyst walls/egg shells.

6.2 OPTIMISATION OF REAGENTS

The concentration of antibodies in a supernatant can vary from approximately 10 µg/ml from the in vitro culture of a hybridoma line up to 1-5mg/ml in polyclonal antisera. It is important to know the optimum concentration of antibody to use in

an ELISA, the titre of the antibody and the minimum quantity of antigen that can be detected using the optimum reagent concentrations in an ELISA test. This information enables the wastage of reagents and effort to be minimised.

6.2.1 Optimisation of Reagent Concentrations for Competitive Inhibition Enzyme Immunoassay (CIEIA)

Introduction

The CIEIA has been described in detail by Bosworth, Brimfield, Naylor and Hunter (1983) and it is critical to this method's performance that reagent concentrations are optimised (Hunter and Bosworth, 1986).

Materials and Methods

Globodera pallida protein (produced by the homogenisation of whole JJ2) was adsorbed onto the solid phase and monoclonal antibody MR8/4.1 mixed with the same protein were the fluid phase reagents. The solid phase binding was developed with alkaline-phosphatase (AP) labelled goat anti-rat IgG (Sigma Chemical Company, Dorset).

Microtitre plates (Dynatech Laboratories, Sussex) were coated at 16 wells per treatment with 10, 1 or 0.1 µg/ml of *G. pallida* protein tested against four concentrations of MR8/4.1 (undiluted, 1/5, 1/10, 1/50) and goat anti-rat IgG (1/100, 1/500, 1/1000, 1/5000).

The three concentrations of *G. pallida* protein were obtained by diluting a 2 mg/ml stock solution with bovine serum albumin dissolved in phosphate-buffered saline containing "Tween 20" at 500 µl per litre (PBS-T). The plates were coated with 50 µl per well and left overnight at 4°C.

A log dilution of 100 µg/ml *G. pallida* antigen in PBS-T was prepared in a second set of microtitre plates. The appropriate concentrations of MR8/4.1 were prepared in PBS-T and mixed with the log diluted protein before incubation at 4°C for 1 hour.

The protein coated plate was washed by five cycles of filling the wells with cold PBS-T for 1 minute and drying. 50 µl of the protein/antibody mixtures were transferred to their corresponding wells on the protein coated plate, mixed and incubated at 4°C for 1 hour; the antigen in the fluid phase competes with the solid phase bound antigen for the monoclonal antibody and the higher the concentration of antigen in the fluid phase the less monoclonal antibody will bind to the solid phase.

The four concentrations of goat anti-rat AP IgG were prepared in PBS-T, added to the washed plates and incubated at room temperature for 30 minutes. Following three cycles of washing and drying, 100 µl of substrate (1mg/ml p-nitrophenyl phosphate in 0.1M ethanolamine buffer, pH 9.6) was added to each well. The enzyme reaction was allowed to proceed for 30 minutes at room temperature and the coloured product measured spectrophotometrically at 405nm with a Titertek Multiscan micro-ELISA reader (Flow Laboratories, Vienna). The absorbance is inversely related to the concentration of antigen in the fluid phase.

Results

The absorbances of each treatment are represented graphically in Fig. 34 as a series of standard curves.

Discussion

The differences between treatments shown in the graphs suggest that each reagent concentration is critical to CIEIA. The slope of the standard curve should be very steep so that variability in absorbance values plotted arithmetically on the Y-axis will not lead to large variability in MR8/4.1 concentration plotted logarithmically on the X-axis. It can be seen in Fig. 34 that a *G. pallida* protein concentration of 10 µg/ml yielded the highest absorbance values and the steepest curves were obtained with goat anti-rat AP concentrations of 1/100 with MR8/4.1 concentrations of 1/5 and 1/10. However, as the conjugated

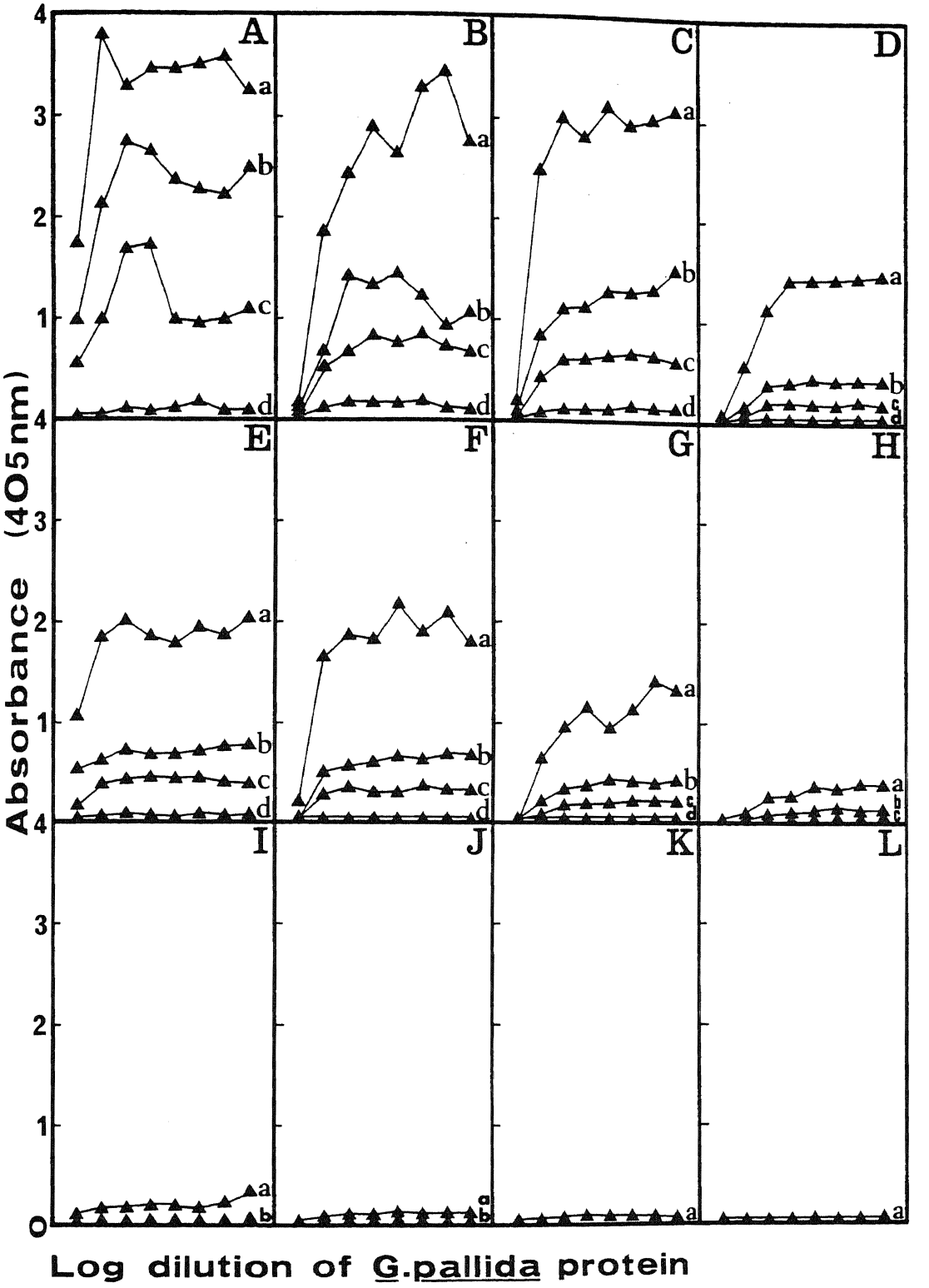


Fig.34 Optimisation of reagent concentrations for CIEIA of G.pallida protein. The coating concentrations of G.pallida protein were 10ug/ml (panels A-D), 1ug/ml (panels E-H), and 0.1ug/ml (panels I-L). Monoclonal antibody MR8/4.1 concentrations were undiluted (panels A,E and I), 1/5 (panels B,F andJ), 1/10 (panels C,G and K) and 1/50 (panels D,H and L). Goat anti-rat IgG concentrations were 1/100 (a), 1/500 (b), 1/1000 (c) and 1/5000 (d).

antibodies are the most expensive reagents in the assay, such high concentrations would be uneconomical. CIEIA with these reagents would have to be a compromise between sensitivity and cost-effectiveness.

6.2.2 Estimating the Titre of Polyclonal Antiserum PC 266

Introduction

The titre of PC 266 was estimated using the standard indirect ELISA protocol used to detect G. pallida antigen.

Materials and Methods

The indirect ELISA protocol:-

1) Coat the micro-ELISA plate with 2 µg/ml nematode protein (or unknown antigen solution) in 0.1M sodium carbonate buffer (pH 9.6) at 50 µl per well. Incubate overnight at 4°C.

2) Wash the plate with 3 cycles of filling each well with PBS-T, leave for 1 minute, blot dry with tissue.

3) Block spare binding sites by the application of 100µl per well of 5% "Marvel" in PBS (PBSM). Incubate for 30 minutes at 37°C.

4) Wash X 3.

5) Apply 1/1000 dilution of PC 266 in PBSTM at 50 µl per well. Incubate at 37°C for 2 hours.

6) Wash X 3.

7) Apply 1/1000 dilution of goat anti-rabbit HRP in PBSTM at 50µl per well. Incubate at 37°C for 2 hours.

8) Wash X3.

9) Apply tetramethylbenzadine (TMB) substrate (Appendix 9) at 100µl per well.

10) After 5 minutes add 2M H_2SO_4 at 30 µl per well and read on the spectrophotometer at 450/690nm.

Results

The results are illustrated graphically in Fig. 35, where absorbance is plotted against the log concentration of PC 266.

Discussion

The optical density decreases linearly with decreasing concentrations of PC 266 and intercepts the X-axis at (log) 4.72, which is a concentration of 1/52,500. The titre of PC 266 is therefore 1/52,500, representing the minimum concentration of PC 266 detectable with a plate coating of 2µg/ml G. pallida and goat anti-rabbit HRP used at 1/1000.

6.2.3 The Effect of Goat Anti-Rabbit HRP Concentration on the Sensitivity of the Indirect ELISA

Materials and Methods

A plate was coated with a double dilution series of 2 µg/ml PC 266 and incubated at 4°C overnight. The following day goat anti-rabbit HRP was used in the indirect protocol (see section 6.2.2) at dilutions of 1/500, 1/1000 and 1/5000 in PBSTM.

Results

The results are displayed graphically in Fig. 36, where optical density is plotted against the log concentration of PC 266.

Discussion

The highest concentration (1/500) of goat anti-rabbit HRP produced the largest optical densities but for all three HRP levels the minimum concentration of PC 266 that was detectable was approximately 1/1000. This represents a PC 266 coating of 2ng/ml. Due to the high cost of goat anti-rabbit HRP a dilution of 1/1000 was chosen for use in the indirect ELISA.

6.2.4 Detection of G. pallida antigen

Introduction

Using PC 266 and goat anti-rabbit HRP at 1/1000 dilution (as used in the indirect ELISA) it would be useful to estimate the minimum quantity of G. pallida antigen that could be detected.

Materials and Methods

A plate was coated with a double dilution series of 10 µg/ml

Fig.35 Estimating the titre of PC266

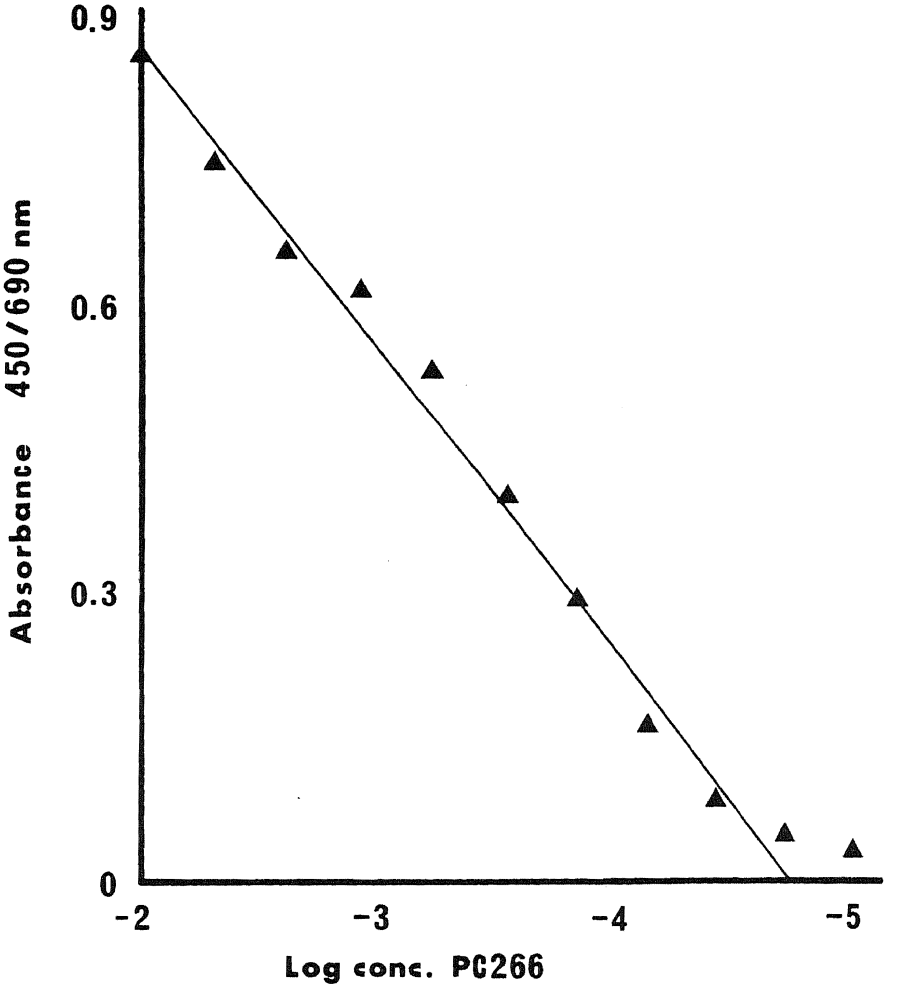
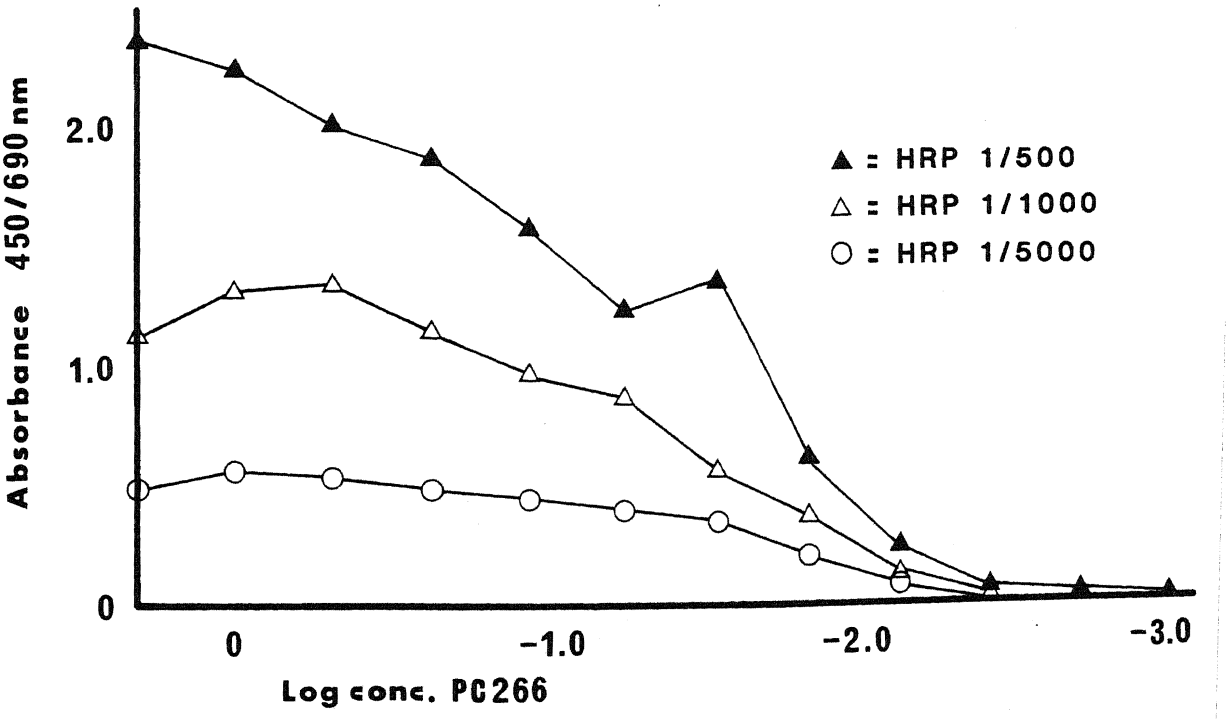


Fig.36 Goat anti-rabbit HRP concentration and the sensitivity of indirect ELISA



G. pallida protein and incubated at 4°C overnight. The following day PC 266 and goat anti-rabbit HRP were used in the indirect protocol (section 6.2.2) at 1/1000 dilutions.

Results

The results are displayed graphically in Fig. 37, where optical density is plotted against the log concentration of G. pallida protein.

Discussion

The curve intercepts the X-axis at log -2.4, which is equal to a G. pallida protein concentration of 3.98 ng/ml. Therefore, the minimum concentration of G. pallida protein that can be detected using the standard indirect ELISA protocol is 3.98 ng/ml.

6.3 THE CHEMICAL RELEASE OF ANTIGEN FROM G. PALLIDA CYSTS

6.3.1 Introduction

Cysts could be disrupted by chemical or mechanical methods in order to release antigen. However, chemical release was favoured because it was more likely to give consistent results and be easy enough to use as part of a diagnostic kit.

6.3.2 Materials and Methods

Sodium hydroxide, hydrogen peroxide, hydrochloric acid, glacial acetic acid, lactic acid, trichloroacetic acid and sodium hypochlorite were all tested for their effect on clean cysts.

A binocular microscope (X 50) was used to observe visible changes in the cysts following the addition of the chemical under investigation.

6.3.3 Results

Only sodium hypochlorite stock solution (Technical grade, approximately 8% available Cl, FSA Loughborough) affected the cyst wall, which had lost its integrity within 11 minutes of hypochlorite exposure (Table 115).

Fig.37 Detection of G.pallida antigen using PC266 and goat anti-rabbit HRP at 1/1000

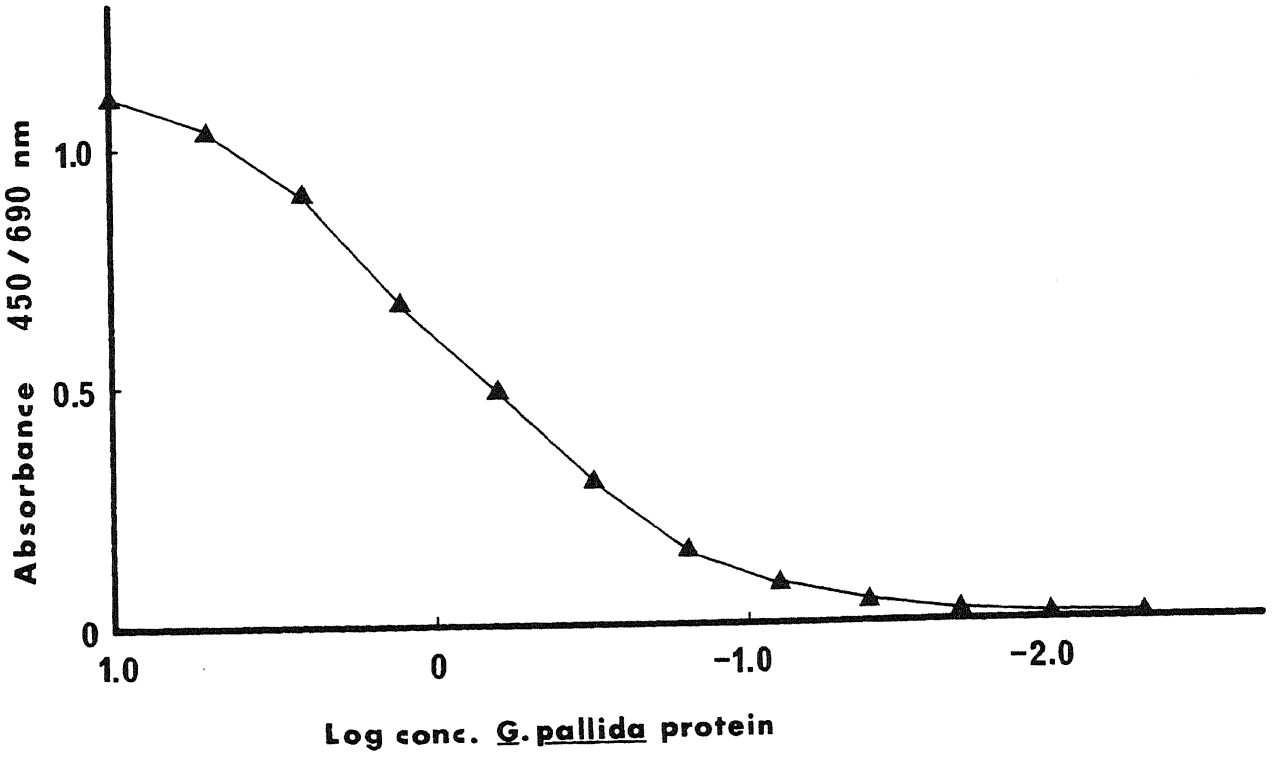


TABLE 115 The effect of NaOCl on the cysts of G. pallida

Time (minutes)	Observation
0	Addition of NaOCl
4	Surface of cysts bubbling
7	Cysts bleached white
11	Cysts starting to split and release eggs
13	Released eggs visible and intact
15	Surface of eggs bubbling - contents leaking out
21	Egg walls starting to collapse. Contents of eggs appear distorted and shrunken
38	Cyst walls fragmented. Empty egg shells visible
70	Egg contents small and compact
90	Little cyst debris left visible

6.4 THE USE OF SODIUM HYPOCHLORITE TO RELEASE ANTIGEN FROM G. PALLIDA CYSTS

6.4.1 Introduction

Esser (1972) has shown sodium hypochlorite to have a destructive effect on many genera of plant parasitic nematodes. Wood and Foot (1975, 1977) found that sodium hypochlorite (1% available chlorine) would destroy PCN cysts in 30-45 minutes, the exact time depending upon ambient temperature. This is comparable with my observations achieved using stock hypochlorite with approximately 8% available chlorine (Table 115).

6.4.2 General Materials and Methods

In the experiments described any antigen released was measured using the indirect ELISA protocol of section 6.2.2. To standardise reaction times the hypochlorite was neutralised by the addition of a small excess of hydrogen peroxide:

$$H_2O_2 + NaOCl \longrightarrow NaCl + H_2O + O_2$$

6.4.3 The Effect of Time of Exposure to Hypochlorite on the Release of Antigen from Cysts

Materials and methods

50 cysts were placed in each of eight plastic vials (1.5ml) to which 1ml of stock hypochlorite was added. The hypochlorite was neutralised with 0.8ml of hydrogen peroxide after 5, 10, 15, 20, 30, 40 or 50 minutes and any antigen released measured using an indirect ELISA.

Results

The results are illustrated in Fig. 38 where optical density is plotted against the length of time of exposure to hypochlorite.

Discussion

The largest quantity of antigen (with an optical density of 0.67) was detected from a digestion period of 10 minutes, after

Fig.38 Effect of duration of exposure to hypochlorite on the release of antigen from cysts

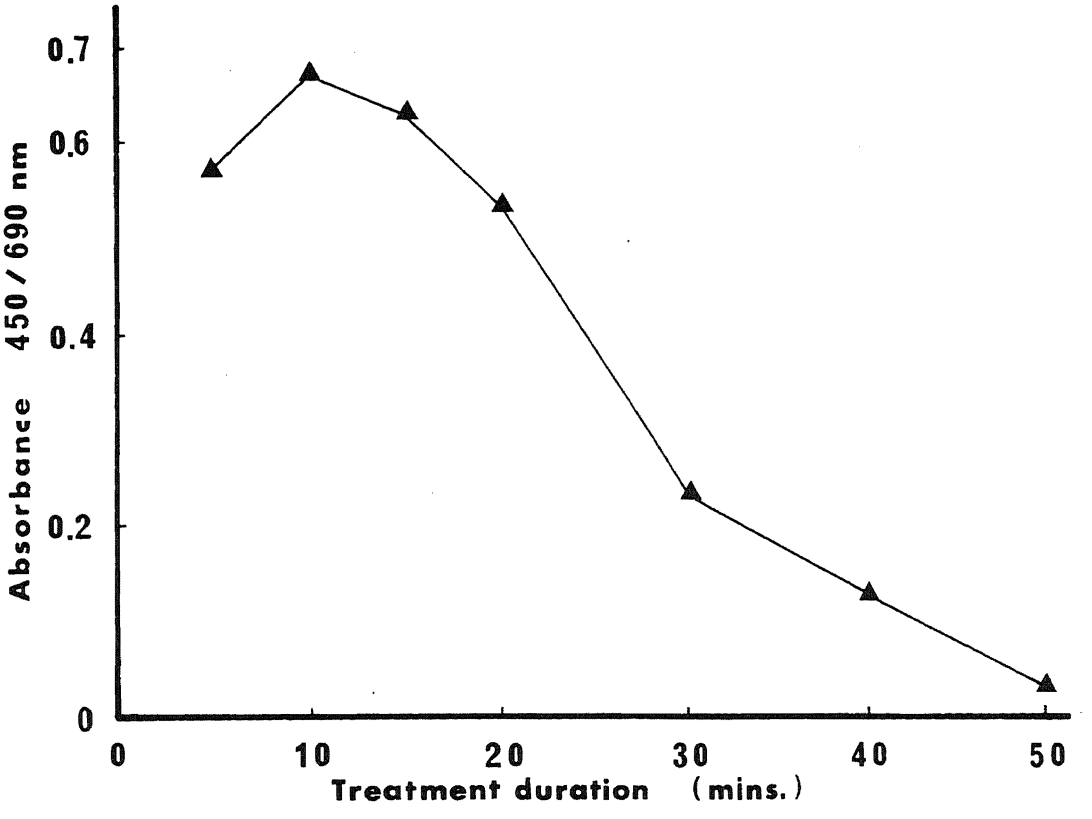
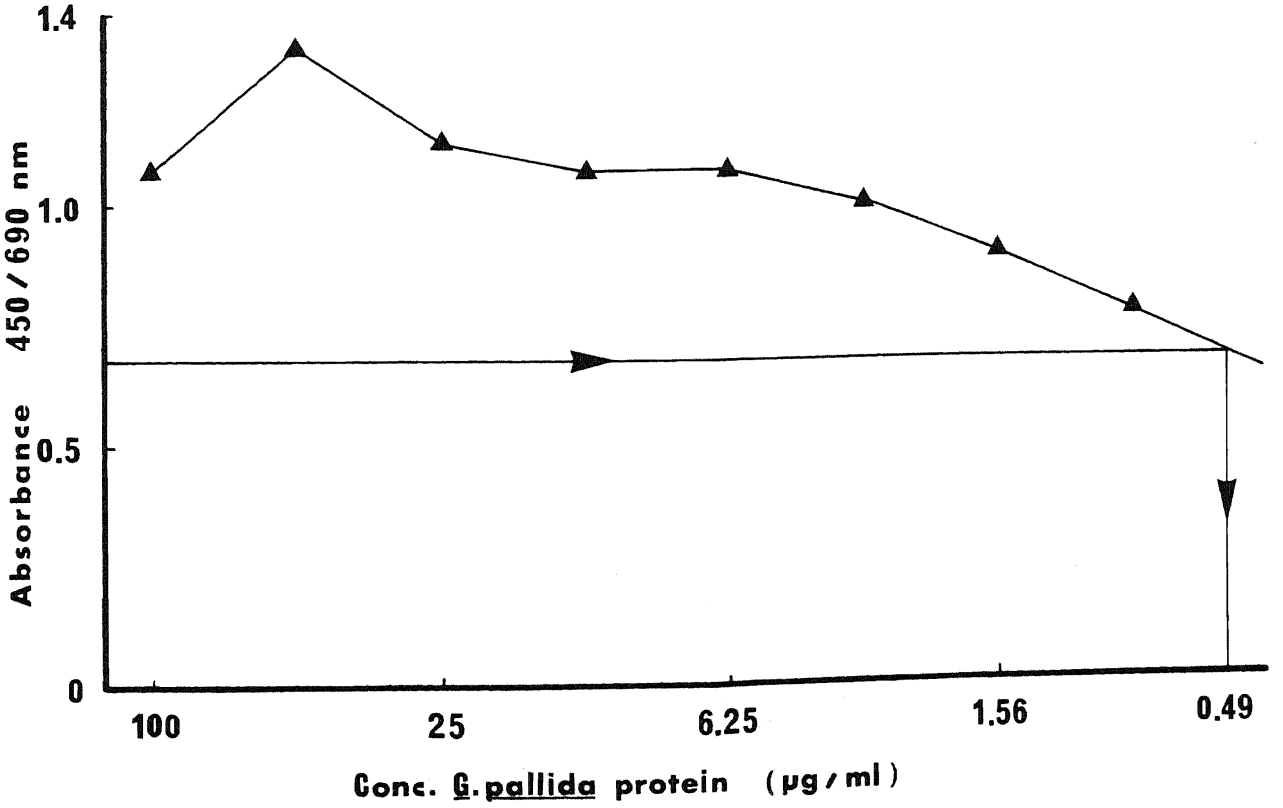


Fig.39 Estimating the quantity of antigen detected after 10 minutes exposure to hypochlorite



which time antigen levels steadily declined.

To estimate the quantity of antigen that has been detected at 10 minutes a standard curve was produced by double diluting a known concentration of G. pallida as a plate coating and then using the indirect ELISA protocol. The optical density was plotted against the log concentration of G. pallida protein (Fig. 39) and the value for an optical density of 0.67 read off the curve. The antilog of -0.31 is 0.49, which represents a G. pallida protein concentration of 0.49 µg/ml.

As the wet weight of a J2 is approximately 100ng and 30% of this is protein (P. R. Burrows, pers. comm.) a single J2 contains approximately 30ng of protein. The egg content of cysts varies from 200 to 500 (Evans and Stone, 1977) so if we assume that our cysts contained 250 eggs then each cyst contained 7.5 µg of protein. With 50 cysts in 1.78ml, if all the protein were released, there would be a concentration of 211 µg/ml. Therefore, treatment with hypochlorite has only enabled us to release and/or detect 0.23% of total nematode protein, which is very low.

6.4.4 The Effect of Hypochlorite Concentration and Exposure Time on the Release of Antigen from Cysts

Materials and methods

Ten cysts were placed in each of 42 plastic vials (1.5ml) and 250 µl of 100, 50, 25, 12.5 and 6.2% by volume of stock hypochlorite solution added. The hypochlorite was neutralised with hydrogen peroxide after 5, 10, 15, 20, 30, 40 or 50 minutes and the antigen concentration in each vial detected using an indirect ELISA. This was repeated for 100, 50 and 25% hypochlorite solution for intervals of up to 325 minutes.

Results

The results are illustrated in Figs 40 and 41, where optical density is plotted against digestion time.

Fig.40 Effect of hypochlorite concentration and the duration of exposure on the release of antigen from cysts

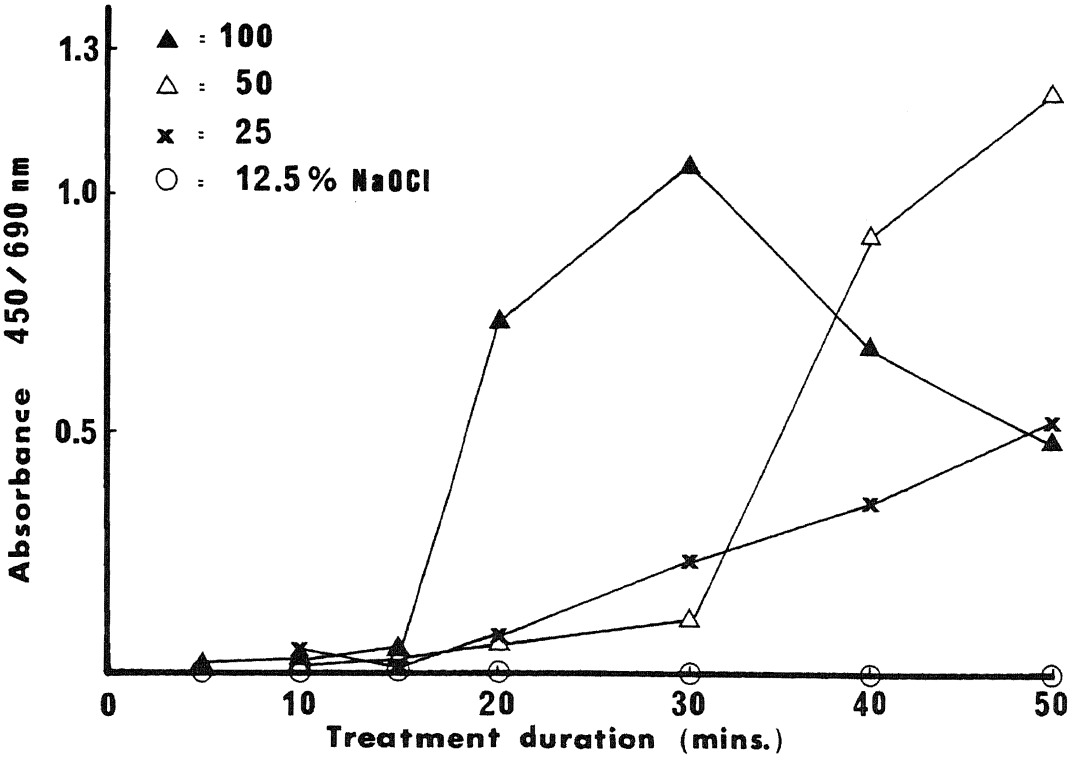
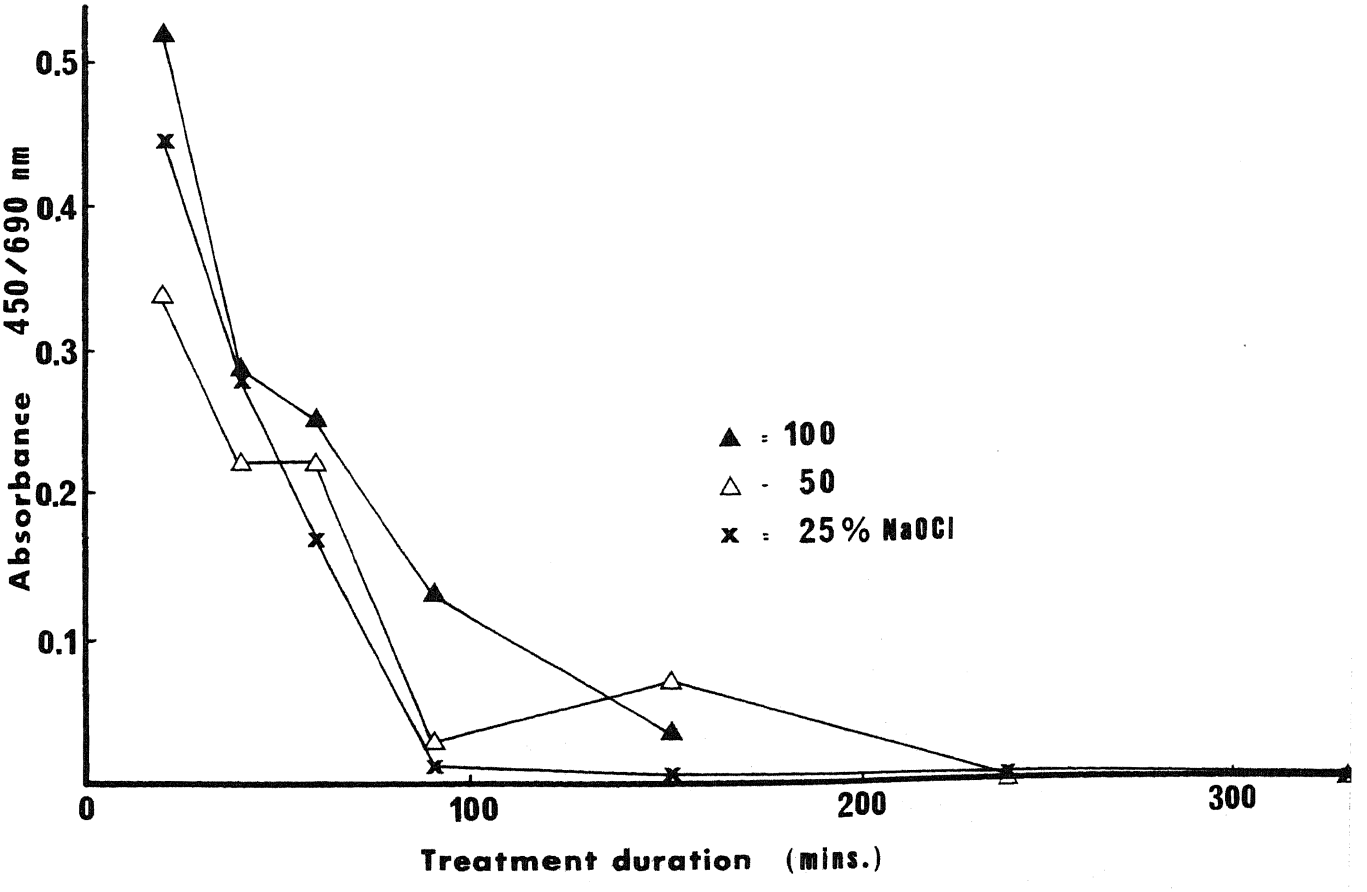


Fig.41 Effect of long periods of exposure to hypochlorite on the release of antigen from cysts



Discussion

In Fig. 40 it can be seen that a 12.5% solution of stock hypochlorite released negligible quantities of antigen. Using 25% hypochlorite, the quantity of antigen released increased gradually; with 50% hypochlorite the rate of increase was faster and most antigen was detected at 50 minutes. The 100% hypochlorite released most antigen at 30 minutes after which period the concentration declined.

Fig. 41 suggests that maximum antigen release for 100%, 50% and 25% occurs in the first 25 minutes of treatment. This conflicts with the results in Fig 40 and implies that either the techniques or the reagents were very variable. Both sodium hypochlorite and hydrogen peroxide are unstable and it is possible that despite keeping them at 4°C and using fresh stock solutions for every test, the activity of these solutions varied between experiments.

6.4.5 General discussion

The use of ELISA in an assay to quantify PCN has great potential. These preliminary investigations have shown that it is possible to release G. pallida protein by chemical treatment and then to detect the antigen using polyclonal antisera and monoclonal antibodies in an indirect ELISA. However, only small quantities of the total available protein were released and results from the system were very variable.

The antibodies used in these experiments were raised to total nematode (J2) homogenates and their specificity is unknown. It would be beneficial to locate the region of the nematode and the epitope which they bind or raise antibodies to specific nematode tissue. For example, a monoclonal antibody raised to denatured (J2) cuticular protein would enable the chemical degradation of cysts to be investigated with the specific aim of exposing JJ2 cuticles.

The effect of hypochlorite on the structure of nematode protein is not known and it would be useful to determine how degradation is achieved. If antibodies could be raised to an epitope that hypochlorite cannot destroy, this would reduce the importance of treatment duration. This would enable a standard (long) treatment period to ensure that all of the cysts in a sample had been fully degraded (advantageous in the treatment of soil samples where the structural, textural and chemical nature of the soil can vary widely). The identification of, and raising antibodies to, a species specific antigen could allow the detection and quantification of both G. pallida and G. rostochiensis in a mixed population.

Further work is still required to optimise the conditions of the assay and possibly increase its sensitivity by using CIEIA rather than indirect ELISA. The variability of antigen release by the chemical degradation of the cyst wall/egg shell could be reduced by accurate determination of the available chlorine concentration in hypochlorite solutions or by using more stable chemicals/enzymes. Mechanical disruption of cysts should also be investigated.

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APPENDICES

APPENDIX 1 Field Trial 1 - cultivar, physiological age, aldicarb treatment and Pi, Pf, Pf/Pi

	Cultivar		
	Pentland Dell	Maris Piper	S.E.D.(1 df)
Pi	333	350	43.1
Pf	576	618	46.0
Pf/Pi	2.03	1.92	0.326

	Day-degrees			
	0	200	400	S.E.D.(2 df)
Pi	311	397	316	52.8
Pf	572	574	646	56.3
Pf/Pi	2.15	1.57	2.20	0.399

	Aldicarb		
	-	+	S.E.D.(1 df)
Pi	336	347	26.9
Pf	646	549	64.5
Pf/Pi	2.23	1.72	0.344

APPENDIX 2 Solar radiation data 1987 and 1988 (Broom's Barn)

WEEK	MJ/m² (1987)	MJ/m² (1988)
1	11.2	13.4
2	19.0	16.7
3	6.9	15.3
4	11.4	17.9
5	36.8	27.2
6	27.3	41.2
7	31.2	41.4
8	37.6	33.3
9	23.5	36.7
10	49.4	59.0
11	64.5	36.6
12	58.8	61.0
13	50.3	53.6
14	63.9	76.3
15	81.6	96.3
16	100.3	80.1
17	139.4	91.9
18	95.0	96.1
19	128.5	79.6
20	96.2	132.2
21	122.8	121.2
22	112.0	110.8
23	90.9	89.7
24	93.2	130.1
25	104.7	130.7
26	113.7	87.9
27	171.3	108.8
28	123.4	109.2
29	55.1	96.1
30	76.8	98.8
31	105.6	104.1
32	84.8	130.2
33	101.3	120.8
34	78.9	87.4
35	64.4	81.8
36	88.8	101.5
37	83.5	65.3
38	70.7	62.1
39	78.2	49.8
40	57.4	64.8
41	49.0	42.3
42	47.9	23.4
43	47.2	34.0
44	21.9	42.7
45	16.2	27.2
46	25.3	27.6
47	15.0	19.5
48	17.2	14.0
49	10.6	14.8
50	7.0	10.6
51	12.5	11.8
52	9.7	17.2

APPENDIX 3 Field Trial 2 - physiological age, planting date, aldicarb treatment and yield

	Day-degrees		
	0	400	S.E.D.(1 df)
Total yield (t/ha)	15.43	13.93	1.122
Ware yield (t/ha)	11.04	10.88	1.174
Percentage ware	61.3	67.3	4.18

	Planting Date		
	1	2	S.E.D.(1 df)
Total yield (t/ha)	14.68	14.68	1.122
Ware yield (t/ha)	11.46	10.46	1.174
Percentage ware	66.8	61.8	4.18

	Estima		M Piper		M Bard		P Dell		SED
	1	2	1	2	1	2	1	2	(3df)
Total(t/ha)	13.93	16.23	24.38	21.65	11.62	13.63	8.80	7.22	2.244
Ware(t/ha)	10.69	12.25	21.53	17.98	8.97	9.34	4.64	2.27	2.347
% ware	66.4	73.2	87.3	81.1	68.2	67.9	45.4	25.0	8.36

	Aldicarb		
	-	+	S.E.D.(1 d.f.)
Total yield (t/ha)	14.57	14.79	0.592
Ware yield (t/ha)	10.81	11.11	0.576
Percentage ware	62.7	65.9	2.01

	Estima		M Piper		M Bard		P Dell		SED
					(3df)				
	-	+	-	+	-	+	-	+	
Total(t/ha)	14.10	16.06	22.63	23.41	13.72	11.53	7.86	8.16	1.794
Ware(t/ha)	10.57	12.38	19.70	19.81	9.91	8.40	3.06	3.85	1.849
% ware	67.2	72.4	85.5	83.0	65.5	70.6	32.6	37.8	6.55

APPENDIX 4 Field Trial 2 - physiological age, aldicarb treatment, planting date and Pi, Pf, Pf/Pi

	Day-degrees		
	0	400	S.E.D.(1 d.f.)
Pi	319	374	30.9
Pf	633	656	41.8
Pf/Pi	2.71	1.95	0.345

	Aldicarb		
	-	+	S.E.D.(1 d.f.)
Pi	338	355	17.1
Pf	630	659	34.2
Pf/Pi	2.36	2.30	0.253

	Estima		M Piper		M Bard		P Dell		
Aldicarb	-	+	-	+	-	+	-	+	SED(3df)
Pi	305	356	296	323	330	325	421	419	49.9
Pf	798	825	738	629	480	550	505	632	76.3
Pf/Pi	3.38	2.82	3.06	2.89	1.53	1.89	1.47	1.62	0.605

	Planting Date		
	1	2	S.E.D.(1 d.f.)
Pi	356	338	30.9
Pf	632	657	41.8
Pf/Pi	2.21	2.45	0.345

APPENDIX 5 Field Trial 2 - physiological age, aldicarb treatment and percentage ground cover duration (PGCD)

Day-degrees	0	400	S.E.D.(1 d.f.)
PGCD	19.9	19.9	1.72

	Day-degrees		
	0	400	
	PGCD		S.E.D.(3 d.f.)
Estima	21.9	21.5	3.44
Maris Piper	30.2	31.3	
Maris Bard	16.2	15.0	
Pentland Dell	11.3	11.9	

	Aldicarb		
	-	+	
	PGCD		S.E.D.(3 d.f.)
Estima	19.4	23.9	2.64
Maris Piper	29.0	32.6	
Maris Bard	16.2	15.0	
Pentland Dell	10.9	12.2	

APPENDIX 6 Field Trial 2 - planting date, aldicarb treatment and plant fresh weight 6 weeks after planting

	Planting Date	Aldicarb		
		-	+	S.E.D.(1 d.f.)
Haulm wt(g)	1	29.4	26.8	2.86
	2	125.2	139.6	13.22
Root wt(g)	1	7.17	7.19	0.465
	2	8.22	8.80	0.692
Total plant wt (g)	1	43.0	40.0	3.39
	2	154.3	168.7	13.48
Total nems/ root system	1	8619	8613	128.4
	2	10012	9845	129.3

APPENDIX 7 Field Trial 3 - planting date, cultivar and Pi, Pf, Pf/Pi

	Planting Date 1	Planting Date 2	S.E.D.(1 d.f.)
Pi	176	173	13.9
Pf	966	1034	61.5
Pf/Pi	12.1	14.5	3.36

	Maris Piper		Pentland Dell		Estima		Maris Bard		S.E.D. (3df)
Planting date	1	2	1	2	1	2	1	2	
Pi	184	143	193	181	169	192	159	176	27.7
Pf	861	920	1224	1071	1154	997	838	934	123.1
Pf/Pi	12.3	25.7	12.0	11.3	13.0	11.6	11.0	9.5	6.72

APPENDIX 8 Field Trial 3 - physiological age, planting date, cultivar and percentage ground cover duration (PGCD)

Day-degrees	0	400	S.E.D.(1 d.f.)
PGCD	36.8	37.5	1.38

	Maris Piper		Pentland Dell		Estima		Maris Bard		S E D (3df)
Day-degrees	0	400	0	400	0	400	0	400	
PGCD	39.4	45.2	37.2	38.7	35.7	32.2	35.0	33.7	2.76

Planting Date	1	2	S.E.D.(1 d.f.)
PGCD	37.4	36.9	1.38

	Maris Piper		Pentland Dell		Estima		Maris Bard		S E D (3df)
Planting Date	1	2	1	2	1	2	1	2	
PGCD	46.0	38.6	38.3	37.6	31.9	36.0	33.4	35.3	2.76

APPENDIX 9 TMB substrate

TMB substrate:-

9ml	distilled water
1ml	1M Sodium acetate pH 5.8
100ul	tetramethyl benzadine (10mg/ml in dimethyl sulphoxide)
2ul	hydrogen peroxide (30%)

APPENDIX 10

Papers submitted in support of candidature

Nematologica 34 (1988)

HAYDOCK, P. P. J.: *Effect of seed tuber physiological age on the yield of potatoes grown in land infested with* Globodera pallida.

In 1987 two field trials were conducted in fen-peat soil at Methwold Hythe, Norfolk, in order to assess whether pre-planting conditioning of seed potato tubers affects the tolerance of potato plants to attack by the potato cyst nematode *Globodera pallida*. After break of dormancy, tubers were conditioned at 13 °C until they accumulated 0, 200 or 400 day-degrees above the development threshold of 4°. In Trial 1, a moderately tolerant cultivar (Maris Piper) was compared with an intolerant cultivar (Pentland Dell) in plots split for aldicarb application (0 v 4.3 kg a.i./ha). Percentage ground cover was measured and destructive growth analysis made at weekly intervals post-emergence. For both cultivars physiological age had a significant effect on tuber yield; 'old' seed usually outyielded 'young' seed. Trial 2 compared cultivars of differing maturity class (early, second early and maincrop) with two planting dates (May 21 and June 11) each plot split for aldicarb application. In Trial 2 physiological age had no significant effect on the yield of any cultivar. The late planting dates gave a short growing season; a longer growth period would have increased yield differences between treatments. At all seed ages Maris Piper outyielded Pentland Dell, which had a lower percentage ground cover than Maris Piper throughout the growing season. Increasing the physiological age of seed produced higher percentage ground cover in early and mid-season for all cultivars and throughout the growing season for Pentland Dell. In Maris Bard and Estima plots 'old' seed senesced earlier than 'young' seed. — *Luton College of Higher Education, Luton, England.*

Tests of Agrochemicals and Cultivars 10 (1989)
Ann. appl. Biol. 114 (Supplement)

SEED TUBER PHYSIOLOGICAL AGE AND THE GROWTH OF POTATO CULTIVARS WITH PARTIAL RESISTANCE TO THE POTATO CYST NEMATODE *GLOBODERA PALLIDA*

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Keywords: Potatoes, potato cyst nematode, *Globodera pallida*, physiological age, tolerance, partial resistance

Introduction

The potato cyst nematode, *Globodera pallida* (Stone), is an increasing problem to the UK potato grower but its multiplication can be partly controlled by growing partially resistant cultivars. However, some of these cultivars are less tolerant of attack than non-resistant cultivars (1). Physiological ageing of seed tubers during storage stimulates shoot growth and hastens crop emergence (2). A well-established, fast-growing crop may be more tolerant of nematode attack. This experiment observed the growth and yield of three partially resistant cultivars compared with two widely grown non-resistant cultivars using seed of two physiological ages (0 and 400 day-degrees above a base temperature of 4°C).

Methods

Graded seed tubers (55-65 g) of clone 12288, Morag, Santé (partially resistant) and Maris Piper and Pentland Dell (non-resistant) were stored at 13°C until break of dormancy. The 0 day-degree tubers were then transferred to a 3°C store; the remainder were kept at 13°C until they had accumulated 400 day-degrees after which they were held at 3°C until planting. The trial site on peaty loam at Methwold Hythe, Norfolk was planted in 1987 with Santé and Maris Piper in order to produce areas of medium or high initial nematode population densities respectively for the 1988 trial. Both areas were divided into three blocks, each containing one randomly assigned replicate of each treatment. Plots were four rows of 12 plants at 30 cm spacing. Rows were 86 cm wide and were marked at each end with three Désirée plants. On 3-5 May seed tubers were placed by hand on the top of small ridges which were then reridged to cover the tubers with soil. A pre-emergence spray of paraquat (Gramoxone 100; ICI) at 1 litre (18% a.i.)/ha controlled weeds. Plots were subsequently hand weeded and rogued. Mancozeb + metalaxyl (Fubol 75; Ciba-Geigy) at 2 kg (67.5 + 7.5% a.i.)/ha was applied in response to ADAS blight warnings. This was tank-mixed with magnesium sulphate at 6 kg/ha on 7 and 23 June. Percentage ground cover was measured at weekly intervals using a viewing frame and expressed as a percentage of total possible ground cover from 8 June until 7 September (Table 1). Two plants were harvested from each plot on 7 July for a growth analysis, including plant dry-weight (Table 1). Nematode population densities were estimated from 30 cores (15 x 150 mm) of soil taken from each plot prior to planting (P_i) and immediately after harvest (P_f). Plots were desiccated with sulphuric acid at 170 litres/ha on 23 September and the middle two rows hand-harvested 26-29 September. Tubers passing over a 40 mm riddle constituted ware yield.

Results and Discussion

The partially resistant cultivars produced higher yields than Maris Piper and Pentland Dell. Yields of Morag, Santé and 12288 were significantly higher than Pentland Dell ($P<0.05$). There were no significant differences in yield between the partial resisters. However, they reduced nematode multiplication rates significantly compared with the non-resistant cultivars ($P<0.01$).

Table 1. Nematode population densities, growth analysis and yield

Cultivar	Day-Degrees	1987 Cultivar	P_i (eggs/g soil	P_f	P_f/P_i	Dry weight /plant (g)	Ground Cover (%)	Total Yield (t/ha)	% Ware
Pentland Dell	0	Santé	90	1192	14.23	80.6	48.1	26.0	45.8
		M. Piper	347	1604	4.80	59.2	45.6	23.5	45.0
	400	Santé	126	858	7.31	104.4	32.3	18.5	31.8
		M. Piper	285	921	3.42	90.6	32.6	23.2	49.5
Maris Piper	0	Santé	123	1002	9.55	90.3	63.6	36.9	71.9
		M. Piper	286	1111	6.45	55.4	58.7	35.2	79.3
	400	Santé	140	706	5.54	89.4	53.6	30.9	58.9
		M. Piper	351	985	3.06	93.6	61.2	36.7	69.5
12288	0	Santé	107	149	1.46	71.4	52.5	44.3	94.7
		M. Piper	275	214	0.99	52.1	48.7	42.2	95.4
	400	Santé	116	146	1.36	86.8	45.1	34.8	82.2
		M. Piper	353	339	0.98	90.9	45.0	37.2	92.4
Morag	0	Santé	117	244	2.38	85.4	54.8	39.7	84.6
		M. Piper	310	262	1.06	76.4	50.2	39.3	82.1
	400	Santé	119	254	2.40	102.1	46.4	33.5	72.1
		M. Piper	256	222	1.04	76.2	39.7	32.5	70.4
Santé	0	Santé	115	204	1.86	88.4	57.4	40.5	83.8
		M. Piper	351	254	0.78	82.8	54.2	40.7	89.2
	400	Santé	98	235	3.01	115.8	54.7	41.9	80.3
		M. Piper	273	264	1.00	86.9	46.2	34.8	79.9
SED (4 d.f.)					2.050	18.07	6.92	6.40	9.05

Mean P_f/P_i ratios were 1.20, 1.66, 1.72, 6.15 and 7.44 for 12288, Santé, Morag, Maris Piper and Pentland Dell respectively (SED 1.025 with 4 d.f.). P_f/P_i ratios were greatest in plots with low initial population densities. Plants grown from 0 day-degree (young) seed outyielded those grown from 400 day-degree (old) seed, though not significantly, and plants from young seed had a higher percentage ground cover than those from old seed, but this was only significant for Pentland Dell ($P<0.05$). On 7 July plant dry-weight was greater in plants from old than plants from young seed but not significantly. Physiologically old seed produced plants with faster initial growth rates than young seed, but this advantage was offset by earlier senescence so that, overall, percentage ground cover was greater for young seed, contributing to greater yields with young seed.

Acknowledgements

My thanks to Greens of Soham Ltd for permission to use the site, Mike Russell, Ken Evans, Corinna Flynn for help with field work and Alan Todd for his statistical advice.

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